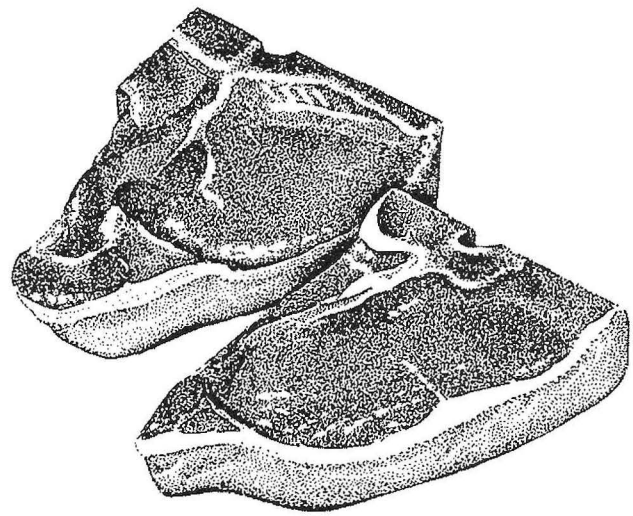
Department of Animal Sciences

Research and Reviews: Meat



December 1999
Special Circular 172
Ohio Agricultural Research and Development Center
In Partnership With Ohio State University Extension

THE OHIO STATE UNIVERSITY OARDC

THE OHIO STATE UNIVERSITY EXTENSION

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Department of Animal Sciences

Research and Reviews: Meat

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Department of Animal Sciences
Ohio Agricultural Research and Development Center
Ohio State University Extension
The Ohio State University

ANIMAL SCIENCES

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Foreword

The Ohio State University Department of Animal Sciences has a rich tradition and has made many contributions to food-animal agriculture in the state of Ohio and beyond. With the dramatic changes that are occurring in all segments of food-animal agriculture, we are challenged to be at the cutting edge to best serve society.

The department is in the process of developing a strategic plan to shape the areas of extension/outreach/engagement, research, and teaching on which the department will focus in the future. The department has accomplished much in the strategic planning that has already been performed. Working with the Animal Sciences Visionary Team, composed of faculty members and industrial partners, however, we will finalize our strategic plan. Without question, there will be continued focus in this department on production efficiency of food animals (cattle, pigs, poultry, and sheep). Other areas that the department is giving strong consideration to are socially responsible and environmentally compatible food-animal management, safety and quality of food-animal products, and understanding the regulation of genes to help in controlling the efficiency of production and the quality and safety of the products that we harvest from food-producing animals. Our department is in the process of hiring faculty members to pursue some of these new areas of study. Indeed, we in land-grant institutions are facing the same challenges that the production segments of agriculture are facing — we need to make wise changes in order to maintain our productivity and efficiency. We also need to remain flexible, so that we can continue to make changes in the future to best serve our stakeholders. The extent to which we continue to serve as we have in the past and to what extent we focus on new areas will be the primary goal of the Animal Sciences Visionary Team. New faces will continue to appear in the *Research and Reviews*, along with changes in the descriptions of the activities of existing faculty so that we can make the changes to better serve our stakeholders.

A question we are frequently asked is to what extent do we intend to expand the scope of the Department of Animal Sciences beyond food-animal production. We have had programs in the equine science area for several years and plans are to continue this program. To what extent we expand into other areas, whether it be new areas of food-animal production or other areas such as companion animal sciences, will be a topic of discussion by our Animal Sciences Visionary Team. Regardless of the programs on which we choose to focus or expand, significant changes will be occurring in the Department of Animal Sciences at The Ohio State University. Whatever we do in changing the department, we want to have a program that is very strong in educating the many students who choose our department to pursue their education. Several of the new faculty members will focus on serving students. We believe that if we have faculty who are dedicated to providing high-quality educational opportunities to our students that we will continue to have a strong student body in the Department of Animal Sciences at The Ohio State University.

My first two months at Ohio State have been very stimulating. I am enjoying working with the faculty and staff and having more students on campus for the academic year. My focus will continue to be on the faculty and the staff members of this department because, in order to serve our students and stakeholders in an efficient and effective fashion, we must have faculty and staff members who are functioning in an effective and efficient manner. My secondary focus is on leading the Animal Science Building Initiative to develop facilities that are of similar quality to those of our neighboring land-grant institutions. I look forward to working with the citizens in the state of Ohio in our educational and building initiatives.

James E. Kinder
Chair

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Contents

Faculty

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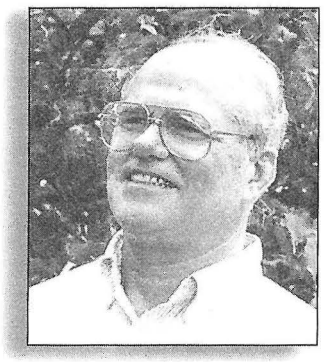
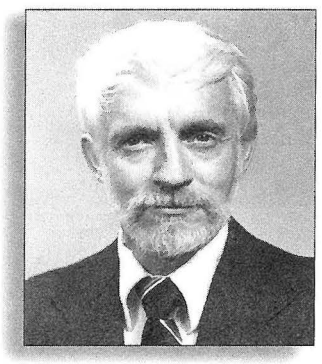
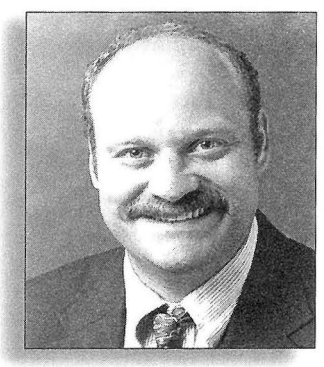


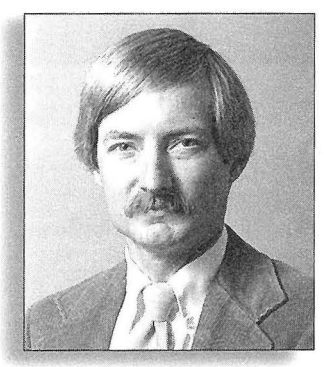
F. R. Allaire, Professor, Columbus. Offers a capstone course where students, working in teams, learn to manage change that serves a client's vision. Works with the Agroecosystems Management Program to inform and facilitate knowledge management networks to support farmers in their pursuit of systemic change in their respective enterprises and communities. Works to have animals add value to community life and land.



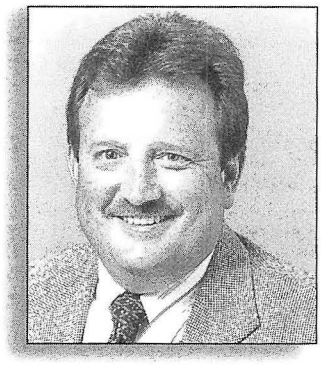
W. L. Bacon, Professor, Wooster. Dr. Bacon's main research focus is avian reproduction. The effects of environmental lighting on semen quality and quantity, and the control of photorefractoryness in the male turkey are being studied. The effects of environmental lighting on circulating hormones at the initiation of photostimulation, and the ovarian-pituitary relationship during the ovulatory surge in the female turkey are also being studied. In female Japanese quail, the effect of yolk precursor lipoprotein concentration on lipid composition of the precursor, and metabolic rate of the precursor is determined.



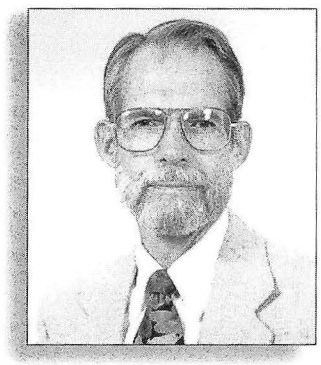
S. L. Boyles, Associate Professor, Columbus. Dr. Boyles is responsible for the state beef cattle education outreach program. His Extension program includes coordinating the activities of the OSU Extension Beef Team and conducting local education workshops. Dr. Boyles' research program emphasizes improved forage utilization through grazing strategies and hay storage systems. Dr. Boyles also is working with commodity organizations on improving beef cattle marketing programs.



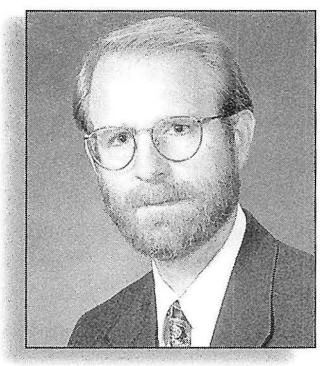
M. E. Davis, Professor, Columbus. Dr. Davis' teaching duties include: Data Analysis and Interpretation for Decision Making (AS 260), Principles of Animal Improvement (AS 320), Research Methods in Animal Genetics I and II (AS 820.02 and 820.04). Research responsibilities include genetics research with the beef herd at the Eastern Ohio Resource Development Center and emphasize studies of postweaning feed conversion, twinning, selection for IGF-I, and marker/QTL associations for growth and body composition in beef cattle. Dr. Davis also is Director of the Animal Genetics Lab, which conducts blood and NA typing for parentage verification for several of the major beef cattle breed associations.



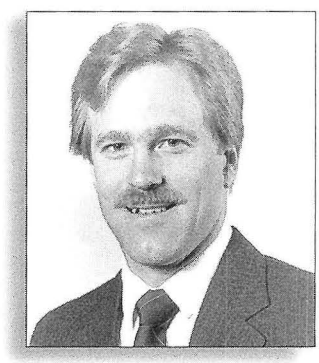
M. L. Day, Associate Professor, Columbus. Dr. Day's research program in reproductive physiology emphasizes the study of puberty, postpartum reproduction, and estrous synchronization in cattle. He teaches the Beef Production and Management and Introductory Animal Sciences courses and advanced reproduction for graduate students. He serves as Faculty Supervisor of the OSU Beef Center.



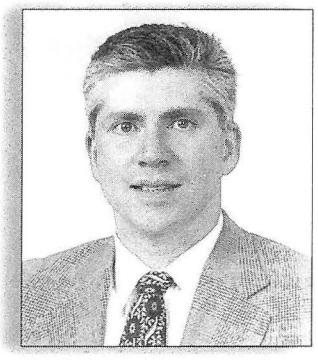
B. A. Dehority, Professor, Wooster. Dr. Dehority's teaching responsibilities include a course in Rumen Microbiology taught every other year during the summer quarter at Wooster, and he advises graduate students. His research interests are in the area of rumen microbiology, including the role of fungi in the rumen, development of MPN procedures for counting rumen bacteria and fungi, the isolation and characterization of rumen bacteria responsible for the breakdown of forage structural carbohydrates, factors affecting protozoal numbers, and specificity of gastrointestinal protozoa, as well as various other specific studies in rumen microbiology.



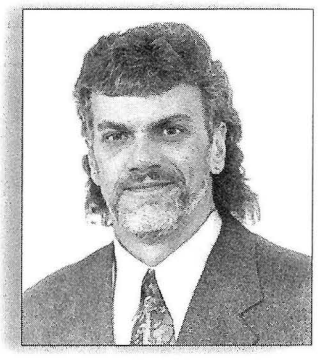
M. L. Eastridge, Professor, Columbus. Dr. Eastridge has Extension and research responsibilities in dairy cattle nutrition and serves as the Coordinator of the Extension Program in Animal Sciences and faculty supervisor for the Waterman dairy facility in Columbus. He conducts educational programs in the area of nutrition. Research includes the impact of fats and feed additives on animal performance and milk composition, and study of optimum fiber in diets for lactating cows. Teaching responsibilities include advising graduate students and co-teaching and serving as leader for an applied dairy nutrition course designed primarily for veterinary students.



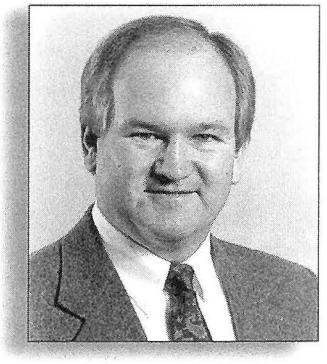
J. L. Firkins, Associate Professor, Columbus. Dr. Firkins' research activities include evaluation of by-products as fiber sources and fat and protein sources for dairy cattle. He studies how these feeds and feeding combinations affect the site of nutrient digestion and efficiency of microbial protein synthesis in the rumen of cattle. Dr. Firkins teaches AS 330, Principles of Animal Nutrition; AS 530, Comparative Animal Nutrition; and AS 730.02, Research Techniques in Animal Nutrition.



F. L. Fluharty, Research Scientist, Wooster. Dr. Fluharty's responsibilities include conducting research in beef cattle and sheep nutrition. His primary research areas are determining the effects of energy and protein intake on animal growth and carcass composition and the nutritional requirements of stressed feeder calves. His research includes work with ruminal microbiology and digestion, as well as cattle and sheep performance studies. He currently is conducting research to determine the effects of nutrition and genetics on animal growth and meat tenderness and the effects of early-weaning beef calves on subsequent feedlot performance and carcass composition. He also teaches AS 540 (Feedlot Management).

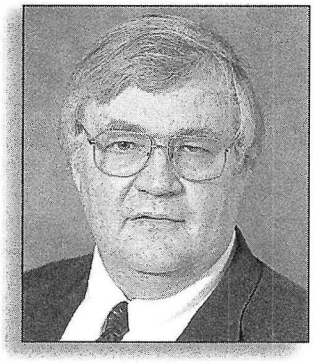


J. S. Hogan, Associate Professor, Wooster. Dr. Hogan's research is in the area of bovine mastitis: hygiene procedures to reduce bovine intramammary infection; relationships among normal and transit teat skin bacterial flora; and milk quality enhancement. He also conducts research relative to the development of a mastitis vaccine, and he teaches the undergraduate lactation course.

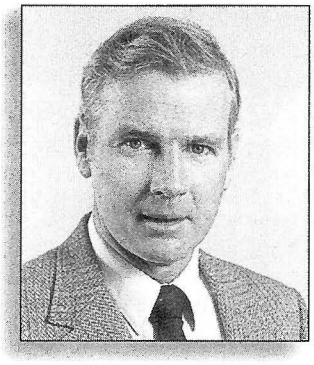


K. M. Irvin, Professor, Columbus. Dr. Irvin's research focuses on swine genetics. Primary consideration is made to the combination of population genetics and molecular genetics. Teaching responsibilities include Principles of Genetic Improvement; Application of Genetic Improvement to Swine; Population Genetics I and II; Advanced Swine Production; Current Issues in Animal Sciences, Capstone and Third Writing Course; Seminars; Independent Studies; and Internships. Extension functions include presentations, allied industry and producer interactions.

C. Johnston, Professor, Wooster. Dr. Johnston's research interests are in the areas of modification of non-milk ingredients for inclusion in milk replacers for cattle and sheep, and dietary macromolecular absorption by cattle and sheep.

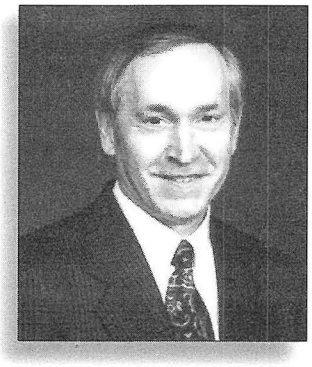


J. E. Kinder, Professor and Chair, Columbus. Dr. Kinder, along with the Associate Chair, Dr. Joy Pate, provides the leadership for administering the various programs in the Department of Animal Sciences. Dr. Kinder also supervises graduate students and conducts research in the area of hormonal regulation of the reproductive function. The focus of his research program is on hormonal regulation of sexual maturation and the reproductive cycle of cattle. He also has an active research program in developing practical technologies to control reproductive cycles of cattle.

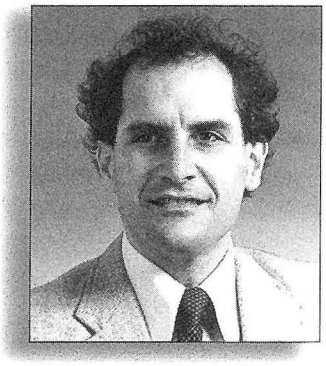


R. C. Kline, Associate Professor, Columbus. Dr. Kline's responsibilities include teaching the horse courses: AS 201, AS 271, and AS 541. His Extension activities include conducting eleven state-wide events each year for the 4-H Program, writing horse materials for both youth and adult programs, and answering the daily requests for information from the horse industry. He oversees the University horse herd and its use for classes and research. Present research involves equine behavior and reproductive physiology in horses.

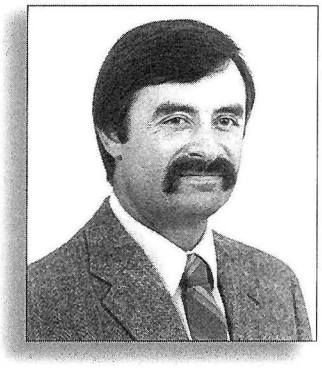
C. L. Knipe, Associate Professor, Columbus. Primary responsibilities include processed meat extension activities for the Ohio meat industry. He is also involved in research and teaching and has a joint appointment with the Department of Food Science and Technology. In addition to providing technical assistance to small and large companies, within Ohio and nationwide, his extension activities have focused on Hazard Analysis and Critical Control Point (HACCP) training and implementation assistance for Ohio meat plants. Dr. Knipe's research interests are identification of processing methods which optimize the functional quality of pork, identification of processing procedures which limit shelf-life and/or safety of meat products, including shelf-life determination of such products, and maximizing the functionality of high-collagen meat raw materials. He advises graduate students in Meat Science and teaches Animal Science 550 (Meat Processing).



J. D. Latshaw, Professor, Columbus. Dr. Latshaw's teaching responsibilities include an introductory course in animal nutrition and one in poultry science. Also, he teaches half of the second nutrition course and half of a course combining nutrition and physiology in support of reproduction. His research interests include documenting all nutrient deficiencies and excesses in broiler chicks and examining the use of energy by birds.

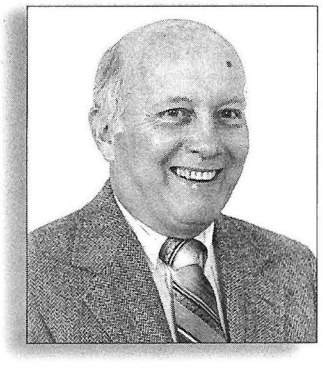


M. S. Lilburn, Associate Professor, Wooster. Dr. Lilburn's research focuses on different aspects of avian nutrition and avian embryonic development. His teaching responsibilities are AS 830.05, a graduate vitamins course, and AS 830.03, a graduate course in proteins. Dr. Lilburn also advises students on the graduate level.

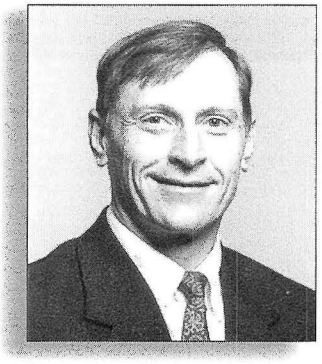


S. C. Loerch, Professor, Wooster. Dr. Loerch's primary research responsibility is in beef cattle nutrition, including effects of controlling intake on feedlot performance and proportion of carcass lean and fat, use of extended grazing and corn as alternative feeds for wintering beef cows, and nutritional strategies for stressed feeder calves. He supervises the OARDC Beef Center and the cow herd at the North Appalachian Experimental Watershed Branch in Coshocton. He teaches an undergraduate practical nutrition course and a graduate-level advanced ruminant nutrition course.

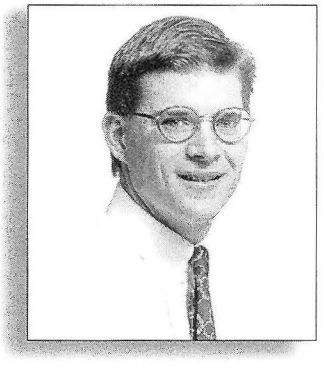
D. C. Mahan, Professor, Columbus. Dr. Mahan's research responsibilities involve evaluating the nutritional requirements and feeding programs of swine at various stages of production, with primary emphasis on the sow and weanling pig. Nutritional areas of investigation include vitamin E and selenium, sodium and chloride requirements of young pigs, dietary protein and energy levels for the gestating and lactating sow, and the evaluation of carbohydrate and energy sources for the weanling pig. He teaches undergraduate courses in Animal Growth and Development and Feeds and Feeding and a graduate course in Mineral Nutrition.



K. E. McClure, Assistant Professor, Wooster. Dr. McClure's research interest is ruminant nutrition with emphasis on forage utilization. Research efforts include grazing experiments with legumes and cool-season grasses using lambs. The objective is meat production that maximizes lean and minimizes fat for health-conscious consumers. Strategies that involve grazing and/or comparative supplemental energy, protein, vitamins, and minerals that are adaptable to the sheep enterprise are a primary objective. Emphasis is also directed to the use of the corn plant and other forages in the basal diet of the ewe flock and breeding rams to economically meet their nutritional requirements. Extension participation includes phone consultations, forage-related farm visits, and meeting with producer groups.

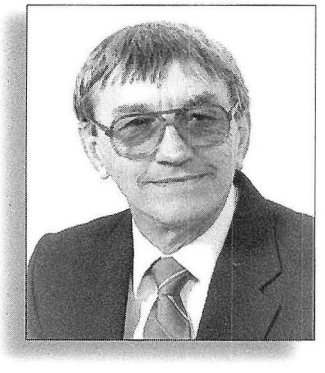


D. L. Meeker, Associate Professor, Columbus. Dr. Meeker's primary responsibilities are in Extension, although he is also involved in research and teaching. He is Coordinator of the Ohio Pork Industry Center, which coordinates expertise from various disciplines to facilitate the profitable and environmentally responsible production of wholesome pork. The Center is an outreach activity of OSU Extension. Dr. Meeker's research interests include swine genetics, particularly genetic effects on muscle quality. He teaches Animal Sciences 643, Advanced Swine Production.

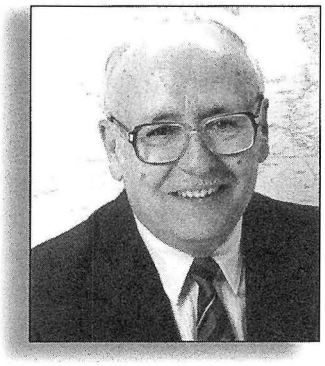


S. J. Moeller, Assistant Professor, Columbus. Dr. Moeller's Extension responsibilities include swine production, management, and genetics/breeding. He works as co-leader of the OSU Swine Educators Team to assist with in-service training and development of comprehensive statewide educational and technology transfer programs important to the Ohio swine industry. His primary research interests are in swine genetics and production management strategies. Teaching responsibilities include Swine Production and Advanced Swine Production.

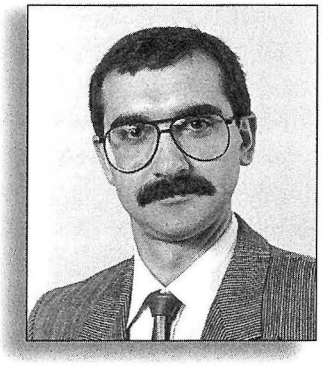
F. V. Muir, Professor, Wooster. Dr. Muir's primary outreach interests are in the areas of the management of layers and broilers, especially the application of computers in the production of eggs and poultry meat. The use of computers to integrate egg production or growth data, feed consumption, poultry house environment, feed formulation, and record keeping are important considerations in remaining competitive. Course responsibility — Commercial Poultry Management.



K. E. Nestor, Professor, Wooster. Dr. Nestor's research interests include population genetics of turkeys and Japanese quail, genetics of disease resistance in the turkey, genetic relationships between growth and reproduction, and genetics of leg strength in the turkey. He advises graduate students and is host to several visiting scholars. Dr. Nestor is a Fellow of the Poultry Science Association and a member of Gamma Sigma Delta.



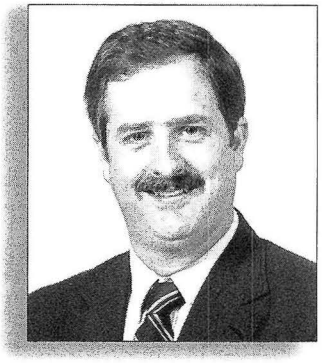
H. W. Ockerman, Professor, Columbus. Dr. Ockerman's teaching responsibilities include Advanced Meat Technology, Laboratory Analysis of Meat Products, Quality Control Interpretation, Global Food and Agriculture, Food in International Agriculture, and Meat Science Seminars, as well as internships and individual studies. He is also involved in international education. His research programs include biochemistry, microbiology, processing, quality, food safety, shelf life, and economics of muscle tissue from slaughter to consumption in all species. Extension duties include short courses, consulting, legal evaluation, and trouble-shooting industry concerns.



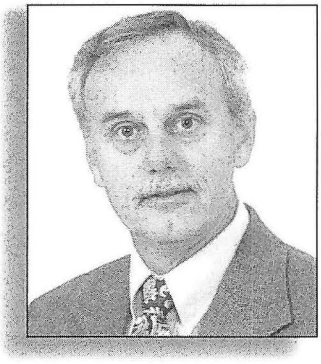
J. S. Ottobre, Professor, Columbus. Dr. Ottobre's research is in the area of reproductive physiology. The primary focus of this research is the regulation of the function of the corpus luteum. He teaches Introductory Animal Science, Reproductive Physiology, and Advanced Reproductive Physiology. Dr. Ottobre has a joint appointment in the Department of Physiology in the College of Medicine.

D. L. Palmquist, Professor, Wooster. Dr. Palmquist's research is in the area of dairy cattle nutrition, including digestive physiology and nutrient utilization of high-energy diets, especially fats, and regulation of milk synthesis and composition. He teaches graduate courses in ruminant nutrition.

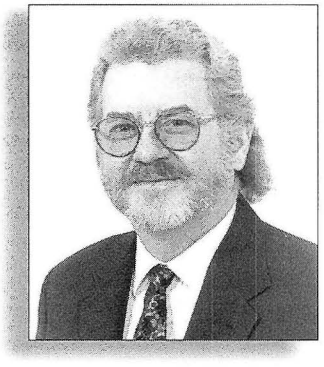
J. L. Pate, Professor and Associate Chair, Wooster. Dr. Pate is a reproductive physiologist specializing in the area of corpus luteum function. Primary research interests focus on the regulation of luteolysis, prostaglandin production by the corpus luteum, the interactions between the immune system and the reproductive system, and nutritional/metabolic effects on fertility. She teaches Physiology of Reproduction and Advanced Reproductive Endocrinology.



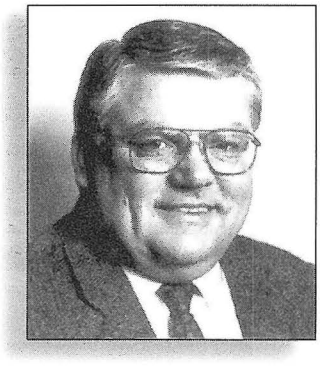
W. F. Pope, Professor, Columbus. Dr. Pope's primary research interests are in embryonic mortality in swine. Secondary investigations are examining factors affecting fertilization, estrous cycle control, uterine secretions, and isoforms of the estradiol receptor. His teaching responsibilities include the core physiology course (310) and reproductive physiology (410). Extension duties include working closely with commercial sheep producers through field days and site visits.



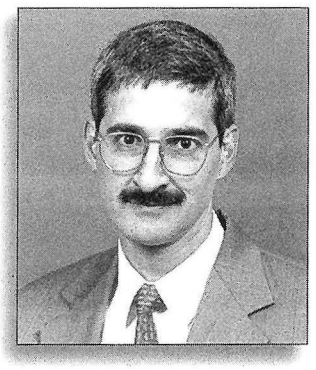
F. L. Schanbacher, Professor, Wooster. Dr. Schanbacher's research interests are in the areas of physiology, biochemistry, and molecular biology of bovine mammary development and milk protein synthesis. Studies are focused at whole animal, cellular, and molecular biology levels for synthesis and secretion of milk protein, mammary cellular growth and development, and growth regulation. He teaches the advanced course in Physiology of Lactation.



K. L. Smith, Professor, Wooster. Dr. Smith's research is in the area of diagnosis, therapy, and control of bovine mastitis in dairy herds; natural factors of disease resistance associated with the bovine mammary gland; and environmental and nutritional factors associated with increased mastitis in dairy herds. He advises numerous M.S. and Ph.D. students.



P. W. Spike, Associate Professor, Columbus. Dr. Spike has appointments in Extension and teaching, including Extension responsibilities in youth work (4-H and FFA), genetics, and management. His teaching duties include dairy cattle evaluation, dairy herd management, and dairy farm management. He also coaches the dairy cattle judging teams and advises the Buckeye Dairy Club.



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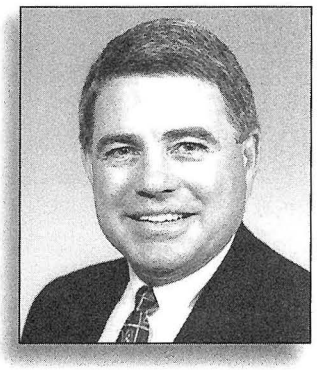

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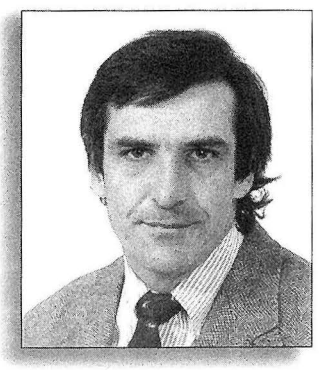

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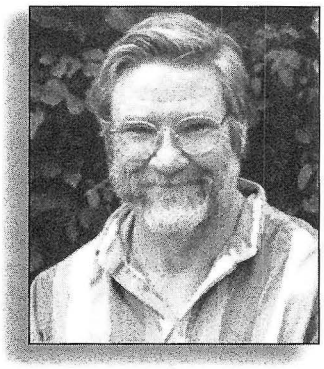
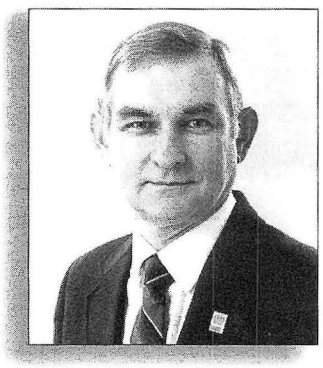
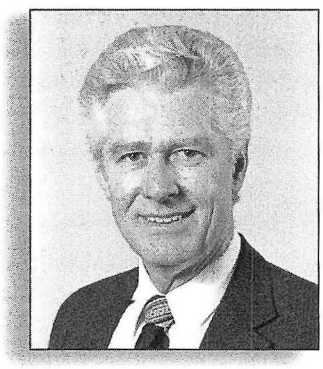


M. P. Wick, Assistant Professor, Columbus. Dr. Wick's research focuses on the role of sarcomeric proteins in the mechanisms controlling skeletal muscle growth and development and meat quality. Teaching responsibilities include AS 355.01, Principles of Meat Science; AS 620, Applied Animal Molecular Genetics; and AS 868, Molecular Biology Techniques.



L. B. Willett, Professor, Wooster. Dr. Willett's research interests are in the area of food, feed, and animal safety, which is the study of the movement and effects of hazardous or toxic materials in food-producing animals. He also studies the physiological adaptive changes that occur in calves immediately after birth. His teaching responsibilities are in the graduate toxicology courses, and he advises graduate students in physiology and toxicology. Dr. Willett also advises independent study students in a collaborative effort with the College of Wooster.



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The Relationship of the Sarcomeric Architecture to Meat Tenderness

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Abstract

Researchers continue to study the muscle cell to elucidate its role in meat tenderness. An increased knowledge of sarcomere architecture is essential to understanding the relationship of this contractile unit to muscle growth and development and ultimately meat tenderness. The central event in growth and development of the muscle cell is the precise assembly of the sarcomere, a highly ordered and complex array of numerous proteins. The biological mechanisms controlling the organization of the sarcomere have been extensively studied. This review, excerpted from an invited review article submitted to *1999 Recent Research Advances in Food and Agricultural Chemistry,* will discuss the major components in the sarcomere with an emphasis on those components thought to contribute significantly to meat tenderness.

Introduction

Animal protein excels in palatability, amino-acid balance, and biological value; it is an important nutritional source sought by developed, developing, and undeveloped countries. The harvesting of meat animals with less subcutaneous, intermuscular, and intramuscular fat has necessitated the simultaneous production of more tender musculature, since fat reduction can decrease texture and lubrication during mastication with a resultant perception of decreased tenderness. This production change has necessitated a more thorough knowledge of postmortem changes that occur during the conversion of muscle to meat. Furthermore, an understanding of muscle growth and development is crucial to the efficient production of meat animals for a growing world population that desires a supply of palatable and nutritious protein. Knowledge of sarcomeric architecture is essential to understanding the relationship of this basic contractile unit of the cell to muscle growth, development, and meat tenderness.

Scientists and consumers both recognize that tenderness is one of the most important sensory attributes of meat. The meat industry has responded to consumer desire for tenderness using a variety of techniques including aging, electrical stimulation, mechanical, chemical, and enzymatic tenderization of muscle. Physiologists, muscle-cell biologists, and biochemists continue to investigate the muscle cell to learn more about tenderness indices. In addition, the central event in the growth and development of the muscle cell is the accumulation of the constituent proteins of the sarcomere.

The Sarcomere

Knowledge of effects of the structural and regulatory proteins of the sarcomere on the physical properties of meat is important to tenderness de-

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terminants. These proteins can affect the amount of muscle contraction and sarcomere length, and the structures that they comprise vary in the susceptibility to proteolytic degradation, which ultimately affects tenderness. This review addresses our current understanding of the role of myofibrillar and cytoskeletal proteins in the sarcomere on meat tenderness and quality.

Each skeletal muscle cell is a filamentous, multinucleated structure composed of up to 1,000 fibers termed myofibrils. The sarcomere, the basic contractile unit of muscle (Figure 1), is that portion of the myofibril between two adjacent Z-disks. Photomicrographs suggest that striated muscle sarcomeres are composed of a highly ordered array of two sets of filaments, thin filaments anchored at one end in a structure, the Z-disk, and interdigitating thick filaments (Squire, 1997). The Z-disk is the boundary for the sarcomere and is oriented perpendicular to the thin filaments and the long axis of the myofibril. The thin filaments of the sarcomere, composing the I-band, are isotropic; i.e., they do not refract birefringent light. The thick filaments called A-bands are anisotropic and refract birefringent light (McCormick, 1994). The A-bands are bipolar and possess projections, heads that bind to actin along either end of the filament.

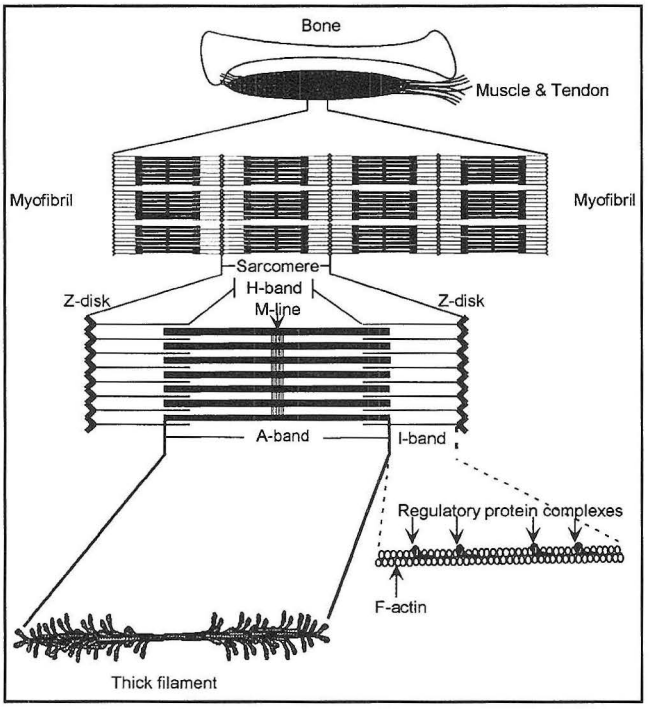

Figure 1. Schematic relating the biochemical components to the microscopic structure of muscle tissue. The ultrastructure of the sarcomere showing the myofibrillar composition of muscle segment between two Z-bands.

The center of the A-band, the "bare-zone," contains a zone free of these projections. The M-line, composed of many "myosin-binding proteins," bisects the bare zone perpendicular to the axis of the sarcomere. The lightly stained zones in the center of the sarcomere, where the thick and thin filaments do not overlap when the sarcomeres are in the relaxed state, are the H-zones. That portion of the H-zone free of thin filaments in the I-band when the sarcomere is fully shortened during contraction is the pseudo-H-zone. Oriented parallel to the Z-disk within the I-band are faint appearing N-lines. A cross section orientation of the sarcomere reveals various patterns of actin, myosin, or actin and myosin filaments. In addition to myofibrillar proteins, the sarcomere contains a third filament system consisting of cytoskeletal proteins that provide lateral and longitudinal support that stabilize the contractile apparatus of the muscle cell, discussed later.

Myosin Structure

Sarcomeric myosin is an asymmetric molecule, 160 nm in length and 2 to 10 nm wide. Myosin is composed of two 220 kDa heavy chains (MyHCs) and four light chains (LCs) ranging from 17 kDa to 22 kDa (Lowey and Risby, 1971). The entire molecule is approximately 160 nm in length. The heavy chains interact to form two distinct domains; a pair of low-salt-soluble globular heads (S1), 15 nm long and 9 nm wide, and a low-salt-insoluble a-helical coiled-coil rod domain. The rod domain is approximately 150 nm long and 2 nm in diameter and is composed of the C-terminal 1100 amino acids of the MyHC. The myosin rod is a two-stranded coiled-coil motif characterized by two parallel amphipathic α-helical chains that are intertwined into a left-handed superhelix termed an α-helical coiled-coil (McLachlan and Karn, 1982; Crick, 1953)

The globular head or S-1 domain of the MyHC is an actin-activated ATPase responsible for myosin's molecular motor function. Based on X-ray crystallographic analysis (Rayment *et al.*, 1995), the three-dimensional structure of the S-1 was determined and a significant revision to our under-

standing of the mechanisms of muscle contraction was proposed (Rayment, 1996).

The C-terminus of the MyHC contains amino-acid sequence elements responsible for the unique solubility and assembly properties of myosin. Furthermore, sequence elements in the C-terminus contribute to the association of a family of myosin-binding proteins (MyBPs). These proteins, along with titin, contribute to the proper assembly of the thick filament. An understanding of these elements will increase our knowledge of the mechanisms controlling muscle growth and development.

Location

Myosin, the most abundant protein in muscle, plays the predominant functional role in many aspects of muscle growth, development, force generation, and tenderness. Recent studies employing molecular genetic techniques have increased our understanding of the contribution of both the N-terminal and the C-terminal sequences in the sarcomeric muscle MyHC to myosin's unique functions. Elucidation of myosin's structure / function relationship will contribute not only to technologies that will enable the future production of high-quality processed-meat products but also to an understanding of the biological mechanisms involved in muscle growth, development, and meat tenderness.

Myosin is a protein possessing multiple functions integral to muscle contraction, force generation, muscle development, and production of high-quality processed meats. These functions include Mg^{++}-ATPase activity, molecular motor activity, actin binding, differential salt solubility, thick filament assembly, and intermolecular interactions with constituent proteins in the sarcomere.

Myosin is expressed as a series of developmentally regulated and tissue-specific isoforms or proteins with *almost* identical structures (Taylor and Bandman, 1989; Gordon and Lowey, 1992; Bandman *et al.*, 1982; Tidyman, 1996; Moore *et al.*, 1992). Different muscles are composed of different proportions of muscle-fiber types. The differential accretion of certain myosin isoforms in specific muscle fiber types is well-documented (Rosser *et al.*, 1997; Rosser *et al.*, 1998; Schiaffino and Salviati, 1997). The functional diversity of myosin isoforms is not understood completely. However, the tenderness and the functionality of meat have been correlated with the proportion of muscle fiber type (Xiong, 1994).

Tenderness

Although the degree of overlap between the thick and thin filaments (Figure 1) contributes significantly to the tenderness of meat, it is the enzymatic degradation of proteins in the Z-band which contributes most significantly to the tenderness of meat. The mechanism of muscle tenderization is not completely understood. Sarcomeres in postmortem muscle that have been aged show disruption of the I-band that is believed to contribute to the tenderization of meat. Calpains, calcium activated proteases, are believed to play a role in the proteolysis of the I-band. *In vitro* calpains demonstrate specificity for myosin. However, little or nor enzymatic degradation of myosin is known to occur *in vivo* in postmortem muscle.

Actin

Actin, the predominant protein of the thin filament, along with actin-binding proteins have been identified in organisms across the evolutionary spectrum. The actomyosin complex that forms soon after death is the major contributing feature of rigor mortis. It is the resolution of rigor that contributes to meat tenderness. However, the actomyosin complex is not broken during normal postmortem tenderization or aging. It has not been possible to crystallize the actomyosin complex. However, high-resolution electron microscopy has demonstrated that the actomyosin rigor interface is extensive, involving interaction of a single myosin head with regions on two adjacent actin monomers. A number of hydrophobic residues on the opposing faces of actin and myosin contribute to the main binding site. This site is flanked on three sides by charged myosin surface loops that form predominantly ionic interactions with adjacent regions of actin. Hydrogen bonding is likely to play a significant role in actin-actin and actin-myosin interactions since many of the contacts involve loops (Milligan, 1996).

The Z-Disk

Z-disks are the boundaries of the sarcomere and aid in maintaining the sarcomere in register in the

myofibril. They serve as an anchoring plane of the thin actin filaments; they link titin and actin filaments from opposing sarcomere halves in a lattice connected by a-actinin and contain desmin. Titin, the most abundant and largest muscle cytoskeletal protein, occurs in thin elastic filaments that run parallel to the thick and thin filaments of the sarcomere. Nebulin, another cytoskeletal protein, appears to run parallel to and in close association with the thin filaments. Desmin, a small cytoskeletal protein occurs within and between Z-disks of adjacent sarcomeres to maintain lateral association between sarcomeres. Studies employing protein interaction analysis demonstrate that two types of titin interactions are involved in the assembly of a-actinin into the Z-disk. These scientists indicated that titin interacts by means of a single binding site with the two central spectrin-like repeats of the outermost pair of α-actinin molecules. Spectrin, a cytoskeletal protein composed of a series of repetitive protein motifs, plays a role in maintaining the bi-concave disk morphology of red blood cells. Another function that occurs within the central Z-disk is the interaction of titin with multiple a-actinin molecules by means of their C-terminal domains. This activity permits the assembly of a complex of titin, actin, and a-actinin *in vitro* with constraint of the path of titin in the Z-disk likely (Young *et al.*, 1998). Actin filaments from adjacent sarcomeres are anchored in the Z-disk of striated muscles. Each filament overlaps with four filaments from the opposite sarcomere, forming a square lattice that is cross-connected in a zigzag pattern by Z-filaments that appear to consist of a-actinin (Luther *et al.*, 1996).

Young *et al.* (Young *et al.*, 1998) postulated that if titin acted as a regulator for Z-disk assembly, the central Z-disk region of the molecule should contain binding sites for other sarcomeric proteins. This hypothesis is supported by the knowledge that native titin binds to α-actinin (Jeng and S. M. Wang, 1992). In fact, others (Turnacioglu *et al.*, 1996) reported that recombinant fragments of Z-disk titin bind to a-actinin.

The binding of titin to the C-terminal domain of a-actinin appears not to be the only protein interaction that controls the sarcomeric sorting of the molecule (Young *et al.*, 1998). These workers suggest that a second Z-disk specific binding site can be predicted from these observations, which should be important for the proper orientation of the Z-disk. They identified this second binding site of α-actinin as an interaction between the spectrin-like repeats and a single site of titin. Furthermore, they demonstrated that the central Z-repeats of titin can interact equally with the C-terminal domain of α-actinin, similar to the flanking repeats. The assembly of complexes of titin, α-actinin, and actin *in vivo* are controlled by these interactions. Young and co-workers combined these observations with ultrastructural data on the position of the N-terminus of titin and the C-terminus of nebulin to develop a molecular model of titin, actin, and α-actinin within the Z-disk. This model will be the foundation for studies to more fully understand the architecture of the sarcomere (Young *et al.*, 1998).

Regulatory Proteins

Muscle contraction is controlled by the action of a complex of regulatory proteins. In striated muscle, the thin filament consists largely of actin and the actin-binding proteins tropomyosin (Tm) and the troponin (Tn) complex (TnI, TnC, and TnT). The thin filament is responsible for mediating Ca^{2+} control of muscle contraction and relaxation both antemortem and postmortem. Contraction is initiated by the elevation of the intracellular Ca^{2+} concentration. Employing EPR and ST-EPR spectroscopy Li *et al.* proposed that the binding of Ca^{2+} to TnC induces a series of conformational changes, which ultimately release the inhibition of the actomyosin ATPase activity by TnI (Li *et al.*, 1997). In the absence of Ca^{2}+ binding to troponin C, TM blocks the site of filamentous actin responsible for binding myosin.

Tm is a small polypeptide, (284 amino acids), depending on the particular isoform, and dimerizes to form a head-to-tail coiled-coil structure that lies in the major groove of the actin filament. The placement of the Tm dimer is consistent with one of its important roles, which is to help mediate cooperativity of Ca^{2+} activation along the length of the myofilament (Solaro and Rarick, 1998). On skeletal muscle thin filaments (Miki *et al.*, 1998), one Tm covers seven actin monomers. Tm is a rigid rod-shaped protein that binds along the length of the actin filament and is intimately associated with TnI. Employing *in vitro* nanomanipulation, investigators were able to directly measure the stiffness

of single actin filaments with and without the presence of Tm and showed that Tm both stabilizes and stiffens the thin filament (Kojima *et al.*, 1994).

References

Bandman, E., Matsuda, R., Strohman, R. C. Developmental appearance of myosin heavy and light chain isoforms *in vivo* and *in vitro*. *Developmental Biology* 1982, 93, 508–18.

Crick, F. H. C. The packing of α-helices: simple coiled-coils. *Acta Cryst.* 1953, 6, 689–97.

Gordon, D. A., Lowey, S. Distribution of developmental myosin isoforms in isolated A-segments. *Journal of Muscle Research and Cell Motility* 1992, 13, 654–677.

Jeng, C.; Wang, S. M. Interaction between titin and α-actinin. *Biomed. Res.* 1992, 13, 197–202.

Kojima, H., Ishijima, A., Yanagida, T. Direct measurement of stiffness of single actin filaments with and without tropomyosin by *in vitro* nanomanipulation. *Proc. Natl. Acad. Sci. U.S.A.* 1994, 91, 12962–6.

Li, H. C., Hideg, K., and Fajer, P. G. The mobility of troponin C and troponin I in muscle. *J. Mol. Recognit* 1997, 10, 194–201.

Lowey, S. and Risby, D. Light chains from fast and slow muscle myosins. *Nature* 1971, 234, 81–5.

Luther, P. K., Squire, J. M., and Forey, P. L. Evolution of myosin filament arrangements in vertebrate skeletal muscle. *Journal of Morphology* 1996, 229, 325–35.

McCormick, R. J. Structure and properties of tissues. In: *Muscle Foods.* A. W. K. D. M. Kinsman, and B. C. Breidenstein, Ed. Chapman & Hall: New York. 1994.

McLachlan, A. D. and Karn, J. Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacings in muscle. *Nature* 1982, 299, 226–31.

Miki, M., Miura, T., Sano, K., Kimura, H., Kondo, H., Ishida, H., and Maeda, Y. Fluorescence resonance energy transfer between points on tropomyosin and actin in skeletal muscle thin filaments: does tropomyosin move? *J. Biochem.* (Tokyo) 1998, 123, 1104–11.

Milligan, R. A. Protein-protein interactions in the rigor actomyosin complex. *Proc. Natl. Acad. Sci. U.S.A.* 1996, 93, 21–6.

Moore, L. A., Arrizubieta, M. J., Tidyman, W. E., Herman, L. A., and Bandman, E. Analysis of the chicken fast myosin heavy chain family. Localization of isoform-specific antibody epitopes and regions of divergence. *J. Mol. Biol.* 1992, 225, 1143–51.

Rayment, I. The structural basis of the myosin ATPase activity. *The Journal of Biological Chemistry* 1996, 271, 15850–53.

Rayment, I., Holden, H. M., Sellers, J. R., Fananapazir, L., and Epstein, N. D. Structural interpretation of the mutations in the beta-cardiac myosin that have been implicated in familial hypertrophic cardiomyopathy. *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92, 3864–8.

Rosser, B. W., Wick, M., Waldbillig, D. M., Wright, D. J., Farrar, C. M., and Bandman, E. Expression of myosin heavy chain isoforms during development of domestic pigeon pectoralis muscle. *Int. J. Dev. Biol.* 1998, 42, 653–61.

Rosser, B. W. C., Wick, M., Waldbillig, D. M., and Bandman, E. Heterogeniety of myosin heavy chain expression in fast-twitch fiber types of mature avian pectoralis muscle. *Biochem. Cell Biol.* 1997, 74, 715–28.

Schiaffino, S. and Salviati, G. Molecular diversity of myofibrillar proteins: isoforms analysis at the protein and mRNA level. *Methods Cell Biol.* 1997, 52, 349–69.

Solaro, R. J. and Rarick, H. M. Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments. *Circ. Res.* 1998, 83, 471–80.

Squire, J. M. Architecture and function in the muscle sarcomere. *Curr. Opin.Struct. Biol.* 1997, 7, 247-57.

Taylor, L. D. and Bandman, E. Distribution of fast myosin heavy chain isoforms in thick filaments of developing chicken pectoral muscle. *The Journal of Cell Biology* 1989, 108, 533–542.

Tidyman, W. Characterization of the chicken neonatal myoain heavy chain isoform: Differential expression of the chicken fast myosin heavy chain genes during develoment, and in normal and dystrophic adult mucsles. Ph.D. Thesis. 1996.

Turnacioglu, K. K., Mittal, B., Sanger, J. M., and Sanger, J. W. Partial characterization of zeugmatin indicates that it is part of the Z-band region of titin. *Cell Motil Cytoskeleton* 1996, 34, 108–21.

Xiong, Y. L. Myofibrillar protein from different muscle fiber types: Implications of biochemical and functional properties in meat processing. *Critical Reviews in Food Science and Nutrition* 1994, 34, 293–320.

Young, P., Ferguson, C., Banuelos, S., and Gautel, M. Molecular structure of the sarcomeric Z-disk: two types of titin interactions lead to an asymmetrical sorting of alpha-actinin. *Embo J.* 1998, 17, 1614–24.

Filament Assembly Properties of the Sarcomeric Myosin Heavy Chain

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Abstract

Meat is the edible muscle tissue of animals. The sarcomere is the fundamental functional unit of muscle. Growth and development of muscle is the result of the highly ordered accretion and assembly of the constituent proteins in the sarcomere. Primary amino acid sequence elements of the constitutive proteins carry the information necessary for determining the final architecture of the sarcomere. The mechanisms by which the constitutive proteins are assembled and function together to form the sarcomere and produce muscle contraction is just now beginning to be understood.

The predominant protein in the sarcomere, found in the thick filament system, is myosin. In physiological buffers, purified myosin spontaneously assembles into a synthetic thick filament with a dramatic resemblance to the native thick filament. Some of the amino-acid-sequence elements contributing to myosin's assembly properties may also be critical to myosin's solubility functions that are so crucial to the manufacture of high-quality prepared-meat products. This paper, written in August 1998, was presented to the symposium on cell biology at the 56th Annual Meeting of the Poultry Science Association, The Pennsylvania State University, and summarizes recent experimental results contributing to our understanding of the mechanism of sarcomeric muscle myosin assembly.

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Introduction

Striated muscle is found in all animal groups from coelenterates through vertebrates and comprises 80% or more of all muscular tissue (Hickmand, 1970). Muscle comprises nearly 40% of the body mass of most animals. Figure 1 diagrams the salient features of skeletal muscle cellular organization. Skeletal muscle is made of elongated cells or myofibers specialized for contraction. Each myofiber is approximately 100 μm thick and contains up to 1,000 myofibrils, each about 1 to 2 μm thick. In striated or skeletal muscle tissue, myofibrils display a pattern of alternating light (I) and dark (A) bands. The striations arise as a result of the packing arrangement of the filament systems in the sarcomere.

The sarcomere is the basic contractile unit of skeletal muscle and is defined as that portion of the myofibril between two Z-disks. Viewed in two dimensions, the sarcomere consists of two sets of filaments, thick and thin. Thin filaments are composed of filamentous actin (F-actin), each anchored at one end in perpendicularly aligned Z-disks. Associated with the thin filaments are the proteins of the contractile regulation system, tropomyosin (Tm) and troponin (TnI, TnT & TnC), and the giant proteins, titin and nebulin, reviewed elsewhere (Moos *et al.*, 1995). Interdigitating between the thin filaments are bipolar thick filaments, composed almost entirely of the fibrous contractile protein myosin. The fine structure of the A-band and of the various myosin-binding and other filament-binding proteins of native thick filaments is re

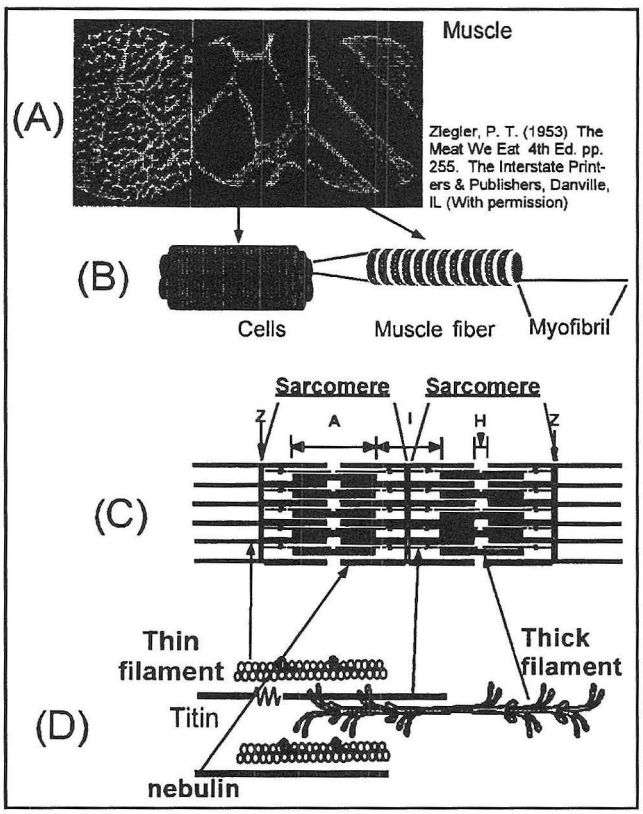

Figure 1. Schematic relating the biochemical components to the microscopic structure of muscle tissue. (A) Cross section of muscle and (B) a longitudinal view of a muscle fiber showing the striated pattern of muscle cells. (C) The ultrastructure of the sarcomere. (D) Magnified view of thick and thin filaments and their associated filament system.

viewed elsewhere (Davis, 1988a; Sjostrom and Squire, 1977; Seiler *et al.*, 1996).

Myosin

Sarcomeric myosins belong to the Type II class of myosin proteins that contain an α-helical coiled-coil rod domain that is involved in the assembly of bipolar thick native and synthetic filaments (Goodson and Spudich, 1993; Cheney *et al.*, 1993). Myosin is a relatively large protein with a molecular mass of about 520 kDa (Figure 2). Each myosin molecule is composed of two 220 kDa heavy chains (MyHCs) and four light chains (LCs), ranging from 17 kDa to 22 kDa (Lowey and Risby, 1971). The entire molecule is approximately 160 nm in length and 2 nm in diameter. The heavy chains interact to form two distinct domains: a pair of globular heads (S1) 15 nm long and 9 nm wide and α-helical coiled-coil rod domain. The rod domain is approximately 150 nm long and 2 nm in diameter and is composed of the C-terminal 1100 amino acids of the MyHC. The myosin rod is a two-stranded coiled-coil motif characterized by two parallel amphipathic α-helical chains, which intertwine around each other into a left-handed superhelix (McLachlan and Karn, 1982; Crick, 1953).

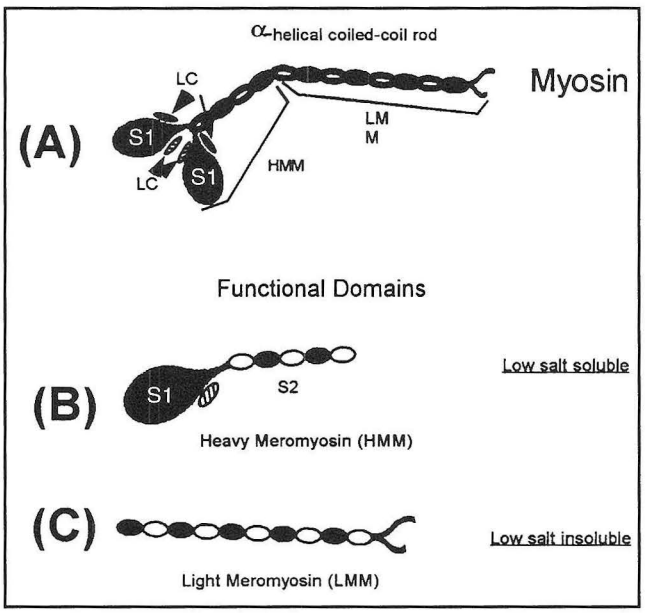

Figure 2. Schematic representation of a sarcomeric myosin molecule. (A) Myosin, a 520-kDa hexameric molecule, is shown along with the common proteolytic fragments, (B) the low-salt soluble heavy meromyosin (HMM), and (C) the low-salt insoluble 130 kDa light meromyosin (LMM).

Structure/function studies on myosin were greatly aided by the discovery that proteolytic enzymes, such as chymotrypsin, trypsin, and subtilisin, cleave myosin into well-defined, high molecular weight fragments (Mihalyi and Szent-Gyorgyi, 1953). Hydrodynamic and electron microscope studies demonstrated that trypsin, chymotrypsin, and subtilisin cleavage occurs predominantly within the rod domain approximately 80 nm from the C-terminus, generating two fragments. The larger fragment, termed heavy meromyosin (HMM), has a molecular weight of 140 kDa, is soluble in low ionic strength buffers, contains the S1 motor domain which is associated with the light chains, has ATPase activity, and binds actin. The 3-D structure of the S1 has recently been determined by X-ray crystallography. Based on this

structure a major update in the mechanism of force generation has been proposed.

The smaller fragment, termed light meromyosin (LMM), has a molecular weight of 80 kDa, is composed of the C-terminal two-thirds of the rod domain, and confers the solubility and aggregation properties to the MyHC. The LMM has been shown to form paracrystalline structures at low ionic strength buffers (Strzelecka-Golaszewska *et al.*, 1985; Harrison *et al.*, 1971; Chowrashi and Pepe, 1977; Atkinson and Stewart, 1991b). Each mole of MyHC associates with 2 moles of light chains (Baba *et al.*, 1984; Collins, 1976; Margossian *et al.*, 1983).

MyHC Rod Domain

The information necessary for myosin's assembly functions is carried in the unique repetitive primary sequence elements of the long α-helical coiled-coil rod domain. The rod consists of two right hand α-helical coils that intertwine around one another forming a left-handed coiled-coil characteristics of the side-chain residues of the amino acids in the primary sequence are repeated every 28 amino acids. Each repeat is composed of 4 seven-amino-acids clusters, called heptads, in which the side-chain residues are similar every seventh amino acid and are labeled *a-b-c-d-e-f-g.*

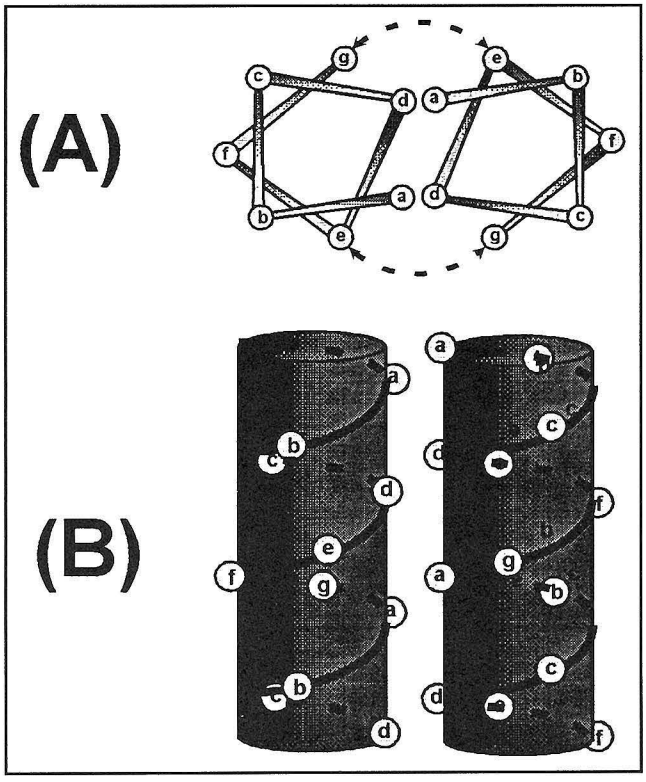

Figure 3. (A) Helical wheel representation of the α-helical coiled-coil motif of the myosin rod. The view is from the N-terminus of the rod domain the heptad positions labeled a through g. (B) Vertical representation of α-helical coiled-coil, 2 heptads.

Fourier transform analysis of the charge characteristics of the primary sequence in each α-helix in the LMM has determined that each 28-residue repeat displays a characteristic sinusoidal pattern of alternating positive then negative charged residues in the *b* and *c* positions of every other heptad. The *b* and *c* positions in the first heptad in every repeat are predominantly occupied by lysine (K) or arginine (R) residues, whereas *b* and *c* positions in the third heptad are occupied by aspartic acid (D) or glutamic acid (E) residues. Thus, the typical repeat is arranged into alternating bands of positive and negative charges (McLachlan and Karn, 1982; McLachlan and Karn, 1983; Parry, 1981), leading to alternating positively and negatively charged zones arrayed along the outer surface of the entire rod (Figure 4).

The observation of this pattern has prompted some investigators to suggest that the reduction in the free energy by the neutralization of opposite charges between myosin rods could provide the energy necessary for myosin assembly if the molecules were staggered by odd multiples of 14 residues (Matsuda *et al.*, 1982; McLachlan and Karn, 1982; McLachlan, 1983; Parry, 1981).

A Mechanism of Assembly

Synthetic Filaments

Myosin is extracted from myofibrils in salt solutions greater than 0.3 M. Purified myosin will precipitate by reducing the ionic strength of the salt solution to < 0.2 M and can be recovered by centrifugation at 5000 X g. If a solution of purified monomeric myosin in 0.6 M NaCl is negatively stained and examined by electron microscopy, no structures or particles are observed. The myosin monomer can be visualized in the electron microscope by the use of shadow-casting, a technique in which a thin carbon/platinum coating is sprayed on the structure (Rice, 1961).

As the ionic strength of a purified myosin solution is lowered from 0.6 M to less than 0.2 M NaCl,

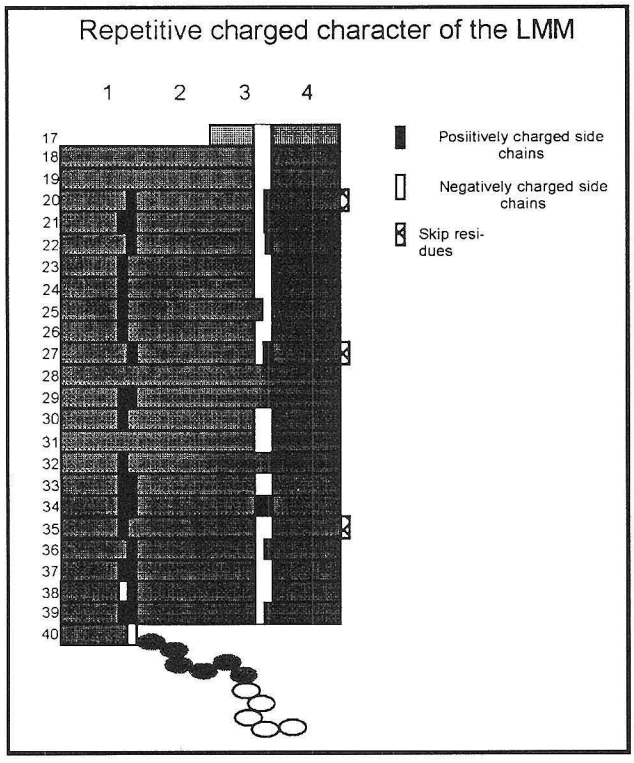

Figure 4. Evolutionary conserved charge character in the LMM. The positions of evolutionary conserved positively charged amino-acid side-chain residues are represented by black rectangles, and negatively charged amino-acid side-chain residues by white rectangles. These clusters are arrayed on the solvent or outer surface of the LMM. The diagram is made by unwinding the α-helix in one of the coiled-coils. Each repeat (28 amino acids) is stacked individually like rungs on a ladder with the next C-terminal repeat aligned underneath. The numbers to the left indicate the repeat number in the α-helix. The LMM is composed of the C-terminal 22-1/2 repeats of the rod from repeat #17 to repeat #40. Each repeat is composed of four heptads, labeled at the top of the figure. Gray boxes indicate neutral, uncharged, or hydrophobic amino-acid side-chain residues. Hatched boxes indicate the position of skip residues in the LMM. Connected ovals at the bottom of the diagram represent the fact that these residues are in a random coil motif.

rod-shaped particles, termed synthetic filaments to distinguish them from native thick filaments, are easily viewed by transmission electron microscopy (TEM) after they have been embedded in a very thin block of uranium acetate. Synthetic filaments display most of the morphological characteristics of native thick filaments and are a good model to study the mechanism of myofibrillogenesis, myosin assembly, and the architecture of native thick filaments (Davis, 1985; Davis, 1988b; Huxley, 1963; Josephs and Harrington, 1966; Katsura and Noda, 1971; Pinset-Harstrom and Truffy, 1979; Pollard, 1975; Reisler *et al.*, 1982).

A putative mechanism of synthetic filament assembly (Figure 5) has been proposed based on the studies of Reisler and Davis (Reisler *et al.*, 1986; Davis, 1988b; Davis, 1986; Davis *et al.*, 1982). Monomers initially assemble into parallel dimers. Dimers assemble into antiparallel tetramers, tetramers into octamers. The octamers finally assemble into a minifilament of 16 molecules, which is the nucleation core for additional assembly and corresponds to the central bare zone of the thick filament. Parallel dimers add on to the tips of the nascent filament in a bipolar fashion until the rate of addition of dimers equals the rate of dissociation of dimers, accounting for the narrow length distribution of synthetic filaments (Davis, 1981; Davis *et al.*, 1982; Davis, 1985; Davis, 1986; Davis, 1988a). Based on these studies, the following assembly mechanism was proposed: as the ionic strength is lowered, myosin monomers interact to form parallel myosin

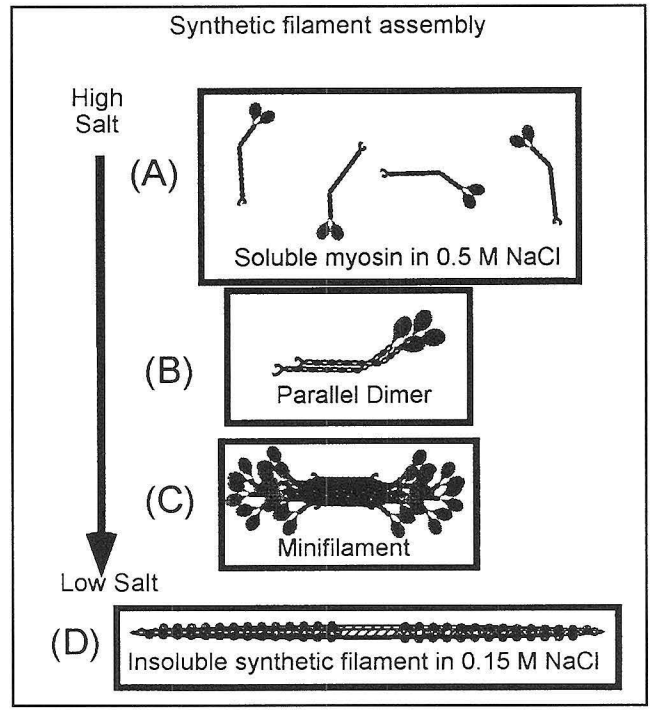

Figure 5. A putative mechanism of myosin assembly. (A) Myosin monomers in high salt spontaneously assemble into (B) parallel dimers, (C) minifilaments, and finally, (D) synthetic filaments as the ionic strength of the solution is reduced.

dimers, which then associate to form a minifilament consisting of myosin associated in an antiparallel arrangement (Reisler *et al.*, 1982; Reisler *et al.*, 1986).

Studies on the effects of anti-rod monoclonal antibody Fab fragments on sarcomeric muscle myosin interactions in low-salt conditions confirmed that regions in the C-terminus of the LMM were responsible for the solubility properties of chicken pectoralis major (PM) muscle myosin (Wick *et al.*, 1997). Unique to these studies were the results that suggest the presence of domains in the N-terminus of the rod controlling the morphology of thick filaments and the architecture of the sarcomere.

The mechanism by which the length distribution of native and synthetic thick filaments is achieved is still a major question in muscle biology. The mechanism has to be consistent with the results of kinetic experiments in which a cumulative property progressively destabilizes the structure of the dimer binding site of the nascent filament. Thermodynamic insight into the mechanism arises from the observation in the electron microscope that native filaments split into three subfilaments in low-salt buffers (Huxley, 1963). The splitting is limited to the tips of the filament where myosin is parallel packed. The central bare zone appears to remain intact. The stable subfilaments in the central bare zone appear to be formed by strong attractive electrostatic interactions, whereas repulsive ionic interactions appear to exist in the regions of the filament in which splits occur. Since filaments formed at pH 8.0 are shorter than filaments formed at pH 7.0, it has been postulated that the repulsive interactions responsible for the splitting of native filaments in low salt are negative in charge (Davis, 1988 b).

Paracrystals

In order to determine the location and presence of amino acid sequence elements in the rod domain of the MyHC and their roles in the mechanism of fibrillogenesis, site-directed mutagenesis of recombinant proteins is becoming the tool of choice. However, a full-length functional hexameric recombinant sarcomeric muscle myosin molecule has yet to be produced *in vitro*. Therefore, investigations into the mechanisms of fibrillogenesis currently employ enzymatically generated and genetically engineered recombinant MyHC rod fragments.

Muscle and nonmuscle myosin rod fragments have been employed to study the intermolecular interactions responsible for filament assembly and solubility (Atkinson and Stewart, 1992; Chowrashi and Pepe, 1977; Chowrashi *et al.*, 1989; Lee *et al.*, 1994; O'Halloran *et al.*, 1990; Ward and Bennett, 1989). Isolated LMM and rod fragments form paracrystals rather than filaments (Ward and Bennett, 1989; Szent-Gyorgyi *et al.*, 1960; Stewart *et al.*, 1989; Parry, 1981; Ishii and Lehrer, 1989; Chowrashi and Pepe, 1977; Atkinson and Stewart, 1991a). In buffers of low ionic strength, the light meromyosin (LMM) fragments of the rod assemble into highly ordered aggregates termed paracrystals, with axial banding patterns based on odd multiples of 14 nm believed to reflect the architecture of the arrangement of myosin in the thick filament (Figure 6). LMM fragments exhibit solubility characteristics that are indistinguishable from full-length myosin. Hence, LMM fragments have proved to be good tools to investigate the assembly properties of sarcomeric myosins. Deletions of the C-terminal portion of a bacterially expressed recombinant LMM generated a fragment that remained soluble in low salt (Atkinson and Stewart,

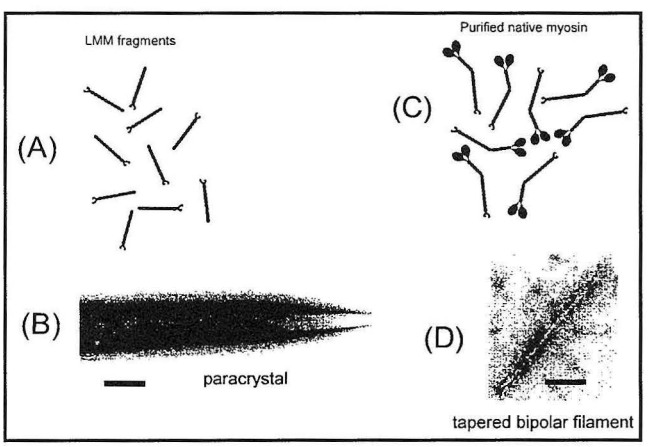

Figure 6. Myosin and rod fragments assemble into aggregates with different morphologies. (A) Illustrates a group of purified myosin molecules. (B) Transmission electron micrograph of a typical negatively stained synthetic filament. The central bare zone is visible in the center of a tapered bipolar aggregate approximately 1.6 μm in length and 15 nm in diameter. (C) LMM fragments are shown to assemble into paracrystals. (D) Transmission electron micrograph shows a typical negatively stained paracrystal. Paracrystals exhibit little or no length or width constraints. The bar = 0.5 μm.

1991a; Sinard *et al.*, 1989; Sinard *et al.*, 1990). The contribution of the C-terminal 100 amino acids to the solubility and N-terminal sequence elements to the assembly of sarcomeric muscle myosin has been demonstrated with bacterially-expressed LMM fragments (Atkinson and Stewart, 1991a). The results of these studies indicated the possibility of sub-domains in the LMM that contribute to various low-energy states of low-salt aggregates of myosin and affect the morphology of native and synthetic filaments.

Implication

MyHC rod fragments assemble into paracrystals, rather than the precisely regulated synthetic filaments, with a narrow distribution of length and width. These observations led to the hypothesis that the bulkiness of the S1 domain of myosin may influence the assembly process. In support of this hypothesis, it was shown that removal of the LC2 from native myosin affected the morphology of synthetic filaments, implicating the myosin head in the mechanism of filament assembly (Chowrashi and Pepe, 1989). We are performing experiments employing bacterially expressed recombinant rod proteins in order to study the sequence elements in the N-terminus of the MyHC involved in myosin's assembly properties. These studies will lead to the discovery of previously undescribed domains in the MyHC. Analysis of the sequence elements in these domains will contribute to our understanding of the intermolecular interactions occurring not only between individual myosin molecules that contribute to thick filament architecture but also between myosin molecules and with other constitutive myofibrillar proteins in determining the architecture of the sarcomere.

References

Atkinson, S. J. and Stewart, M. 1991a. Expression in *Escherichia coli* of fragments of the coiled-coil rod domain of rabbit myosin: influence of different regions of the molecule on aggregation and paracrystal formation. *J. Cell Sci.* 99: 823–836.

Atkinson, S. J. and Stewart, M. 1991b. Molecular basis of myosin assembly: coiled-coil interactions and the role of charge periodicities. *J.Cell Sci.* Supplement 14, 7–10.

Atkinson, S. J. and Stewart, M. Molecular interactions in myosin assembly role of the 28-residue repeat in the rod. *Mol. Biol.* 1992, 226, 7–13.

Baba, M. L., Goodman, M., Berger-Cohn, J., Demaille, J. G., and Matsuda, G. The early adaptive evolution of calmodulin. *Mol. Biol. Evol.* 1984, 1, 442–455.

Bandman, E., Moore, L. A., Arrizubieta, M. J., Tidyman, W. E., Herman, L., and Wick, M. Soc. Gen. Physiol. Ser., Marine Biological Laboratory, Woods Hole, Mass.; The Rockefeller University Press, N.Y.; Vol. 49, p. 129–139.

Cheney, R. E., Riley, M. A., and Mooseker, M. S. Phylogenetic analysis of the myosin superfamily. *Cell Motility and the Cytoskel.* 1993, 24, 215–223.

Chowrashi, P. K., Pemrick, S. M., and Pepe, F. A. LC2 involvement in the assembly of skeletal myosin filaments. *Biochinica et Biophysica Acta* 1989, 990, 216–223.

Chowrashi, P. K. and Pepe, F. A. Light meromyosin paracrystal formation. *J. Cell Biol.* 1977, 74, 136–152.

Collins, J. Homology of myosin DTNB light chain with alkali light chains, troponin C and parvalbumin. *Nature 1976*, 259, 699–700.

Crick, F. H. C. The packing of α- helices: simple coiled-coils. *Acta Cryst.* 1953, 6, 689–697.

Davis, J. S. Pressure-jump studies on the length-regulation kinetics of the self-assembly of myosin from vertebrate skeletal muscle into thick filament. *Biochem. J.* 1981, 197, 309–314.

Davis, J. S. Kinetics and thermodynamics of the assembly of the parallel- and antiparallel-packed sections of synthetic thick filaments of skeletal myosin: a pressure-jump study. *Biochem.* 1985, 24, 5263–5269.

Davis, J. S. A model for length-regulation in thick filaments of vertebrate skeletal myosin. *Biophys. J.* 1986, 50, 417–422.

Davis, J. S. Assembly process in vertebrate skeletal thick filament formation. *Annual Review of Biophysics and Biophysical Chem.* 1988a, 17, 217–239.

Davis, J. S. Interaction of C-protein with pH 8.0 synthetic thick filaments prepared from the myosin of vertebrate skeletal muscle. *J. Musc. Res. Cell Motil.* 1988b, 9, 174–183.

Davis, J. S., Buck, J., and Greene, E. P. The myosin dimer: an intermediate in the self-assembly of the thick filament of vertebrate skeletal muscle. *FEBS Lett* 1982, 140, 293–297.

Goodson, H. V. and Spudich, J. A. Molecular evolution of the myosin family: Relationships derived from comparisons of amino acid sequences. *Proc. Natl. Acad. Sci.* (U.S.A.) 1993, 90, 659–663.

Harrison, R. G., Lowey, S., and Cohen, C. Assembly of myosin. *J. Mol. Biol.* 1971, 59, 531–535.

Hickmand, C. L. *Integrated Principles of Zoology.* 4th Ed. The C. V. Mosby Co., Saint Louis, Mo., 1970.

Huxley, H. E. Electron microscope studies on the structure of natural and synthetic filaments from striated muscle. *Mol. Biol.* 1963, 7, 281–308.

Ishii, Y. and Lehrer, S. Mg^{2+}-paracrystal formation of tropomyosin as a condensation phenomenon: effects of pH, salt, temperature, and troponin binding. *Biophysical J.* 1989, 56, 107–114.

Josephs, R. and Harrington, W. F. Studies on the formation and physical chemical properties of synthetic myosin filaments. *Biochem.* 1966, 11, 3474–3487.

Katsura, I. and Noda, H. Studies on the formation and physical chemical properties of synthetic myosin filaments. *J. Biochem.* 1971, 69, 219–229.

Lee, R. J., Egelhoff, T. T., Spudich, J. A. Molecular genetic truncation analysis of filament assembly and phosphorylation domains of *Dictyostelium* myosin heavy chain. *J. Cell Sci.* 1994, 102, 2875–2886.

Lowey, S. and Risby, D. Light chains from fast and slow muscle myosins. Nature 1971, 234, 81-85.

Margossian, S. S., Bhan, A. K., and Slayter, H. S. Role of the regulatory light chains in skeletal muscle actomyosin ATPase and in minifilament formation. *J. Biol. Chem.* 1983, 258, 13359–13369.

Matsuda, R., Bandman, E., and Strohman, R. C. The two myosin isoenzymes of chicken anterior latissimus dorsi muscle contain different myosin heavy chains encoded by separate mRNAs. *Differentiation* 1982, 23, 36–41.

McLachlan, A. D. Analysis of gene duplication repeats in the myosin rod. *J. Mol. Biol.* 1983, 169, 15–30.

McLachlan, A. D., Karn, J. Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacings in muscle. *Nature* 1982, 299, 226–231.

McLachlan, A. D. and Karn, J. Periodic features in the amino acid sequence of nematode myosin rod. *J. Mol. Biol.* 1983, 164, 605–626.

Mihalyi, E. and Szent-Gyorgyi, A. G. Trypsin digestion of muscle proteins. I. Ultracentrifugal analysis of the process. *J. Biol. Chem.* 1953, 201, 189–196.

Monera, O. D., Kay, C. M., Hodges, R. S. Electrostatic interactions control the parallel and antiparallel orientation of α-helical chains in two-stranded α-helical coiled-coils. *Biochem.* 1994, 33: 3862–3871.

Nguyen, H. T., Gubits, R. M., Wydro, R. M., Nadal-Ginard, B. Sarcomeric myosin heavy chain is coded by a highly conserved multigene family. *Proc. Natl. Acad. Sci.* (U.S.A.) 1982, 79, 5230–5234.

O'Halloran, T. J., Ravid, S., and Spudich, J. A. Expression of Dictyostelium myosin tail segments in *Escherichia coli* : Domains required for assembly and phosphorylation. *J. Cell Biol.* 1990, 110, 63–70.

Parry, D. A. Structure of rabbit skeletal myosin. Analysis of the amino acid sequences of two fragments from the rod region. *J. Mol. Biol.* 1981, 153, 459–464.

Pinset-Harstrom, I. and Truffy, J. Effect of adenosine triphosphate, inorganic phosphate and divalent cations on the size and structure of synthetic myosin filaments. *Mol. Biol.* 1979, 134, 173–188.

Pollard, T. D. Electron microscopy of synthetic myosin filaments. Evidence for cross-bridge flexibility and copolymer formation. *J. Cell Biol.* 1975, 67, 93–104.

Reisler, E., Cheung, P., Borochov, N. Macromolecular assemblies of myosin. *Biophys. J.* 1986, 49, 335–342.

Reisler, E., Cheung, P., Oriol-Audit, C., and Lake, J. A. Growth of synthetic myosin filaments from myosin minifilaments. *Biochem.* 1982, 21, 701–707.

Rice, R. V. Conformation of individual macromolecular particles from myosin solutions. *Biochimica et Biophysica Acta* 1961, 52, 602–604.

Seiler, S. H., Fischman, D. A., Leinwand, L. A. Modulation of myosin filament organization by C-protein family members. *Mol. Biol. Cell* 1996, 7, 113-127.

Sinard, J. H., Rimm, D. L., and Pollard, T. D. Identification of functional regions on the tail of *Acanthamoeba* myosin-II using recombinant fusion proteins. II. Assembly properties of tails with NH_2- and COOH-terminal deletions. *J. Cell Biol.* 1990, 111, 2417–2426.

Sinard, J. H., Stafford, W. F., and Pollard, T. D. The mechanism of assembly by three successive dimerization steps. *J. Cell Biol.* 1989, 109, 1537-1547.

Sjostrom, M. and Squire, J. M. Fine structure of the A-band in cryo-sections: The structure of the A-band of human skeletal muscle fibers from ultrathin cryo-sections negatively stained. *J. Mol. Biol.* 1977, 109, 49–68.

Stewart, M., Quinlan, R. A., Moir, R. D. Molecular interactions in paracrystals of a fragment corresponding to the α-helical coiled-coil rod portion of glial fibrillary acidic protein: Evidence for an antiparallel packing of molecules and polymorphism related to intermediate filament structure. *J. Cell Biol.* 1989, 109, 225–34.

Strzelecka-Golaszewska, H., Nyitray, L., and Balint, M. Paracrystalline assemblies of light meromyosins with various chain weights. *J. Musc. Res. Cell Motil.* 1985, 6, 641–58.

Szent-Gyorgyi, A. G., Cohen, C., Philpott, D. E. Light meromyosin fraction I: A helical molecule from myosin. *J. Mol. Biol.* 1960, 2, 133–42.

Ward, R. and Bennett, P. Paracrystals of myosin rod. *J. Mus. Res. Cell Motil.* 1989, 10, 34–52.

Wick, M., Tablin, F., and Bandman, E. Effects of anti-LMM antibodies on the solubility of chicken skeletal muscle myosin. *J. Food Biochem.* 1997, 20, 379–95.

Dietary Intake of Vitamin E Affects the Peroxide Value of Subcutaneous Lamb Fat

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The Ohio State University Department of Animal Sciences

Abstract

Lipid oxidation is a major problem causing flavor deterioration in meat products. The objectives of this research were to analyze the effects of dietary vitamin E on the lipid oxidation of subcutaneous lamb fat employing a modified iodometric peroxide value (mPV). Lambs were fed *ad libitum*, an all-concentrate diet, formulated to provide 16% crude protein with 15 International Units (IU) (National Research Council recommended level; Control), 300 IU for seven days, or 300 IU for 21 days of supplemental vitamin E (per kg of diet dry matter). The mPV demonstrated significant differences in the lipid oxidation state of animals fed control, 300 IU (seven days) and 300 IU (21 days) of vitamin E ($P < 0.05$). In addition, mPV demonstrated significant differences ($P < 0.05$) in the rate of change in the lipid oxidation state during storage. mPVs demonstrated dramatic increases of the lipid oxidation state of subcutaneous lamb fat on the 11th day in all three treatments. Furthermore, the lambs fed control vitamin E had significantly higher initial PV on day one than those fed 300 IU vitamin E. These results indicate that dietary intake of vitamin E significantly affects the initial lipid oxidation state and the rate of the lipid oxidation of subcutaneous lamb fat and therefore extends the shelf-life of lamb fat.

Introduction

The evaluation of the lipid oxidation state is essential to the palatability of lipid foods. Lipid oxidation is a major problem causing flavor deterioration in meat and its by-products, which adversely affects the shelf life of animal fat. Oxidative rancidity in animal tissue starts to develop almost immediately after meat animal slaughter and carcass fabrication (Gray and Pearson, 1994). Unsaturated fatty acids become oxidized and produce undesirable organoleptic characteristics. The initial step in lipid oxidation is the generation of highly transient hydroperoxides that further degrade into malonaldehyde (MA) and other secondary compounds. Formation of hydroperoxides from unsaturated fats by oxidation has been recognized as the most important pathway for generating precursors of undesirable odors and flavors (Frankel, 1980).

Many antemortem factors potentially contribute to the lipid oxidation state of animal fat, however diet can be one of the most effective ways of inhibiting lipid oxidation. Previous research indicated that supplementing dietary vitamin E, a fat soluble antioxidant, reduces lipid oxidation and increased the shelf-life of lamb meat (Wulf *et al.*, 1995). Current methods of lipid oxidation analyses may contribute to further lipid oxidation and generate by-products that contribute to nonspecific reactions. In addition, these analyses are generally time-consuming and involve harsh conditions, such as steam distillation or oven-heating. Such

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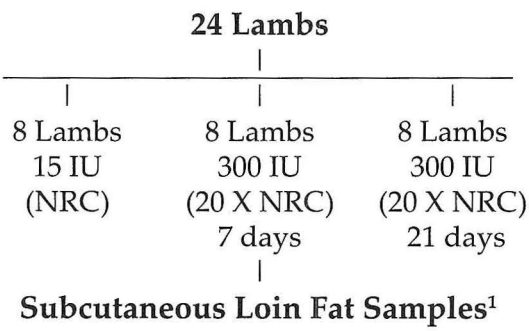
harsh conditions are postulated to contribute to further lipid oxidation (Decker *et al.*, 1998; Shahidi, 1994). Recent research has been conducted establishing a modified iodometric peroxide value (mPV) as a practical method to monitor the lipid oxidation in lamb fat (Li *et al.* 1999, unpublished data). The objective of this research was to employ mPV to investigate the effects of dietary vitamin E on the lipid oxidation of subcutaneous lamb fat.

Material and Methods

Sample Preparation

The experiment was developed as a 3 (15 IU; 300 IU, 7 days; 300 IU, 21 days) x 4 (Days 1, 7, 9 and 11) factorial design (Figure 1). Twenty-four lambs were obtained from The Ohio State University's Ohio Agricultural Research Development Center (OARDC) campus in Wooster, Ohio. They were randomly divided into three groups (eight lambs per group) and fed an all-concentrate diet, offered *ad libitum*, formulated to provide 16% crude protein with one of the following three treatments: 15 IU (National Research Council-recommended; Control), 300 IU (20 X NRC, 7 days) or 300 IU (20 X NRC, 21 days) of supplemental vitamin E per kg of diet dry matter. Carcass fabrication was conducted seven days post-slaughter. Subcutaneous fat from the loin area was removed, wrapped in a Styrofoam meat tray with oxygen-permeable film, and stored at 39 ± 3.6°F (4 ± 2°C) from 1 to 11 days post-slaughter. The lamb fat samples were subjected to the modified iodometric peroxide value on days 1, 7, 9, and 11 post-slaughter.



[1] Stored at 39 ± 3.6°F (4 ± 2°C) in oxygen-permeable film meat trays for days 1 (slaughter day), 7 (fabrication day), 9, and 11 for peroxide values.

Figure 1. Experimental design of different dietary vitamin E treatments on lambs.

Modified Peroxide Value (mPV)

The PV method of analysis was modified from the AOAC (1990) procedure. Briefly, a 50 g sample of unrendered subcutaneous loin fat each was ground in a Waring lab blender (Waring Products Division, Dynamic Cooperation of America, New Hartford, Conn.) for 20–30 s and extracted with 30 mL ice cold (3:2 v/v) acetic acid:chloroform. The extraction was vigorously swirled to distribute the sample and reagents. After the samples were dissolved in the acetic acid:chloroform mixture, 0.5 mL of saturated potassium iodine (KI) (83.2 g solid KI / 40 mL H_2O) was added and mixed vigorously for 10 s. Subsequently, 30 mL deionized water was added, and the solution mixed thoroughly. Color of the upper aqueous layer ranged from pale yellow to bright yellow, with the lower organic layer remaining white. The mixture was allowed to stand for 5 to 10 min. at room temperature then titrated with 0.01 M $Na_2S_2O_3$ (Sigma Chemical, Fair Lawn, N.J.) gradually with vigorous shaking. During the titration 0.5 mL of starch indicator (Starch 1% with chloroform 0.3%, Lab Chem., Inc., Pittsburgh, Pa.) was added. Color of the upper aqueous layer ranged from light purple to dark purple, and the lower organic layer remained white to gray. If the color of the lower organic layer remained yellow, the sample was vigorously swirled and allowed to stand for an additional 10 min. The end-point of titration was established when the color of the upper aqueous layer disappeared. The mPV was calculated employing the following formula:

$$mPV = \frac{(S)(N)(1000)}{W}$$

Where:

mPV = Modified Peroxide Value (meq oxygen/kg fat)
S = mL $Na_2S_2O_3$
N = Normality of $Na_2S_2O_3$ (0.01 N)
W = g of fat

Rate of Lipid Oxidation

The rate of lipid oxidation (m) reflects the onset of lipid oxidation on lamb fat. The rate of lipid oxidation was be calculated by following formula:

$$m = \frac{PV_2\text{-}PV_1}{d_2\text{-}d_1}$$

Where m is the rate of lipid oxidation, meq. of PV/kg/d

PV_2-PV_1 is the difference of peroxide value between two observations, meq./kg

d_2-d_1 is the time period, days

Statistical Analysis

Statistical analysis was completed by statistical software SAS 7.0 for Windows® (SAS Institute, Inc., Cary, N.C.). The significance of the differences was determined by a two-way General Linear Model (GLM).

Results and Discussion

Table 1 demonstrates that there is a significant increase of the lipid oxidation state in the subcutaneous fat obtained from lambs fed all three supplemental vitamin E treatments through day 11 ($P < 0.05$). In addition, the group fed 15 IU vitamin E had much higher initial peroxide value (1.83 min E (1.03 and 0.64 meq./kg), which suggests that meq./kg) on day 1 than those fed 300 IU vita high levels of dietary vitamin E supplement retarded the initiation of lipid oxidation more than the control level of vitamin E treatment. Table 1 also indicates that lipid oxidation significantly increased between day 9 and 11 in the fat from lambs fed all three vitamin E treatments ($P < 0.05$), which suggests high dietary vitamin E treatment reduces the rate of lipid oxidation more than low dietary vitamin E intake. The detailed information is summarized as follows.

Table 1. Effects of Three Different Dietary Vitamin E Treatments on Peroxide Values of Lamb Fat During Storage.[1]

	Peroxide Value, meq/kg		
Days	15 IU	300 IU 7 days	300 IU 21 days
1	1.83[a]	1.03[a]	0.64[a]
7	2.09[ab]	1.09[a]	0.65[a]
9	2.39[b]	1.11[a]	1.00[b]
11	3.40[c]	1.94[b]	1.78[c]

[1] Means followed by different letters within columns are significantly different ($P < 0.05$)

Dietary Vitamin E

Peroxide values demonstrated that there is a significant difference in the lipid oxidation state in the fat obtained from animals fed three different levels of vitamin E ($P < 0.05$) (Figure 2). However, the differences in the mPV between the two 300 IU treatments were not as great as the differences between the control and 300 IU groups. In addition, animals fed 300 IU of vitamin E apparently had lower mPV than those fed 15 IU. These results suggest that a higher level of dietary vitamin E yielded lower mPVs and less oxidation of subcutaneous lamb fat.

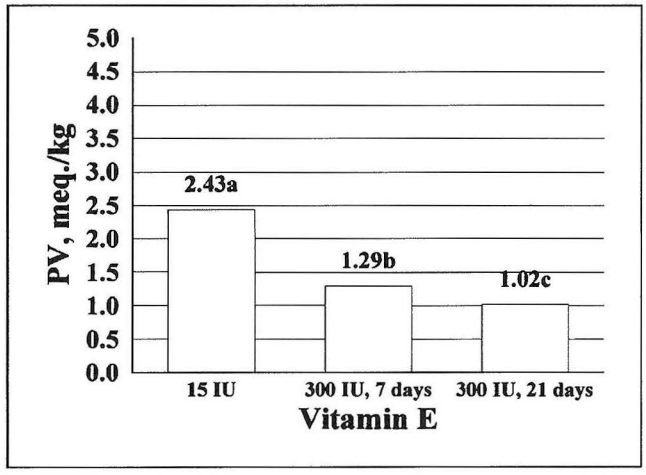


Figure 2. Effects of three different levels of dietary vitamin E on overall peroxide values of subcutaneous lamb fat. IU: International Unit. Means followed by different letters are significantly different ($P < 0.05$). Total observation, n = 192.

Shelf-Life

As the data in Table 1 reflects, there is an increase in the mPV during storage in the fat ob-

tained from lambs fed all three treatments ($P < 0.05$). Figure 3 demonstrates that the control group had significantly higher mPVs than the groups fed 300 IU vitamin E treatments assayed at each storage period. In previous research, Shahidi (1994) indicated that a longer period, required to reach a certain PV, increased effectiveness in inhibiting oxidation. These data suggest that either feeding higher levels of supplemental vitamin E or increasing the supplemental feeding time increases the shelf-life of animal fat through the inhibition of lipid oxidation.

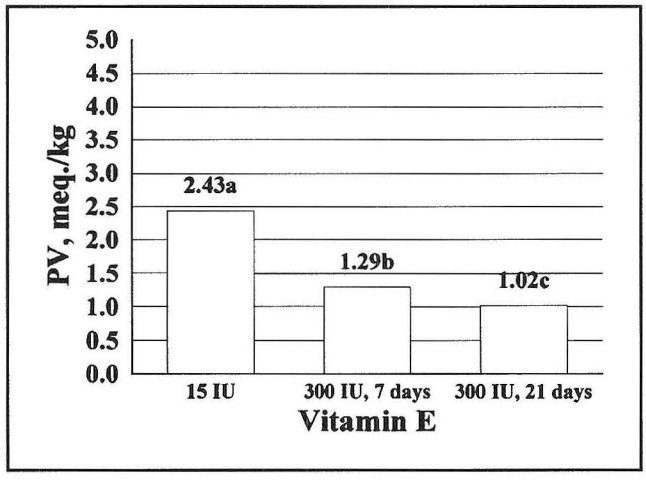


Figure 3. Effects of different levels of dietary vitamin E during storage time on peroxide values of subcutaneous lamb fat. IU: International Unit. Means followed by different letters are significantly different ($P < 0.05$). Total observation, n=192.

Rate of Lipid Oxidation

Figure 4 indicates that mPV significantly increases between day 9 and 11 in the fat obtained from lambs fed on all three vitamin treatments ($P < 0.05$). These data suggest that high dietary vitamin E treatment (300 IU) reduces the rate of lipid oxidation to a greater extent than control vitamin E treatment (15 IU). The equations used to determine the rate of lipid oxidation in the fat from lambs fed the three different dietary vitamin E treatments are shown as follows:

$Y_{15} = 0.50\ X_{15} + 2.39$; where Y_{15} = Peroxide value of 15 IU group and X_{15} = day.
($m_{15} = 0.50$; Intercept = 2.39)

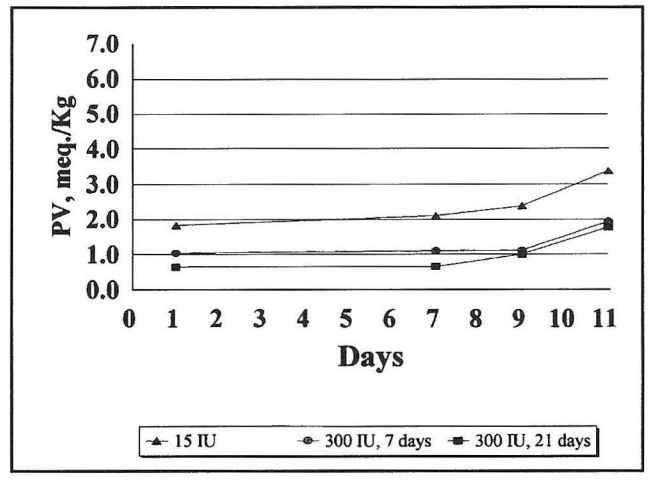


Figure 4. Effects of dietary vitamin E on rate of lipid oxidation of subcutaneous lamb fat. IU: International Unit.

$Y_{300,7} = 0.42\ X_{300,7} + 1.11$; where $Y_{300,7}$ = Peroxide value of 300 IU, 7 days group and $X_{300,7}$ = day.
($m_{300,7} = 0.42$; Intercept = 1.11)

$Y_{300,7} = 0.39\ X_{300,21} + 1.00$; where $Y_{300,21}$ = Peroxide value of 300 IU, 21 days group and $X_{300,21}$ = day.
($m_{300,21} = 0.39$; Intercept = 1.00)

The rate of lipid oxidation is $m_{300,21} \geq m_{300,7} > m_{15,}$ which suggests the fat in lambs fed a higher level and longer period of dietary vitamin E has a greater resistance to lipid oxidation than the fat in the control group. However, the difference between 7 days of 300 IU vitamin E treatment and 21 days of 300 IU vitamin E treatment is not great, which suggests 7 days of 300 IU vitamin E may be sufficient to retard the initiation of lipid oxidation.

Implications

Peroxides are the primary products of lipid autoxidation. Results indicate that vitamin E is effective in inhibiting lipid oxidation. Data also suggest that dietary intake of vitamin E significantly affects the initial lipid oxidation state and the rate of lipid oxidation in subcutaneous lamb fat. Despite the significance between the mPV's at 7 days and 21 days of 300 IU, the difference is not as great as those between 15 IU and 300 IU. This data indi-

cates that the full antioxidant effect of vitamin E can be obtained in as little as 7 days of supplemental feeding which could have a significant economic contribution for the meat animal industry in Ohio. Further evaluation is necessary to determine the optimal time and levels of feeding supplemental antioxidants on increasing the shelf life and palatability of the fat from meat animals.

Acknowledgements

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References

AOAC 1990. *Official Methods of Analysis of the Association of Official Analytical Chemists.* Chapter 4, p. 956. 15th Ed. K. Helrich, Ed. Assoc. of Official Analytical Chemists, Inc., Arlington, Virginia.

Decker, E. A., Chan, W. K. M., and Faustman, C. 1998. TBA as an index of oxidative rancidity in muscle foods. *Proc. 51st Ann. Recip. Meat Conf.* p. 66. American Meat Science Assoc., Kansas City, Mo.

Draper, H. H., McGirr, L. G., and Hadley, M. 1986. The metabolism of malonaldehyde. *Lipids.* 21 (4): 305.

Frankel, E. N. 1980. *Survey on Lipid Science and Technology.* p. 96–98. The 15th ISF Congress, April 27–May 1. International Society for Fat Research, New York.

Fiedler, U. 1974. A coulometric method for the determination of low peroxide values of fats and oils. *J. American Oil Chemists' Society.* 51 (3): 101–103.

Gray, J. I. 1978. Measurement of lipid oxidation: A review. *J. American Oil Chemists' Society.* 55 (6): 539–546.

Gray, J. I. and Pearson, A. M. 1994. Lipid-derived off-flavor in meat — formation and inhibition. In: *Flavor of Meat and Meat Products.* 1st Ed. Shahidi, F., Ed. pp. 117–139. Chapman & Hall, London, U.K.

Mehlenbacher, V. C. 1960. Determination of peroxides in meat and meat products. In: *Analysis of Fats and Oils.* pp. 226–228.

Nawar, W. W. 1996. Lipids. In: *Food Chemistry.* 3rd Ed. Fennema, O. R., Ed., pp. 226, 255–273. Marcel Dekker, Inc., New York.

Shahidi, F. 1994. Assessment of lipid oxidation and off-flavor development in meat and meat products. In: *Flavor of Meat and Meat Products.* 1st Ed. Shahidi, F., Ed. pp. 247–266. Chapman & Hall, London, U.K.

Witte, V. C., Krause, G. F. and Bailey, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* 35: 582–585.

Wulf, D. M., Morgan, J. B., Sanders, S. K., Tatum, J. D., Smith, G. C., and Williams, S. 1995. Effects of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts. *J. Animal Sci.* 73: 399–405.

Evaluation of Lipid Oxidation in Animal Fat

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Abstract

Lipid oxidation is a major contributor to flavor deterioration in meat products. A modified peroxide value (mPV) method was compared to the 2-thiobarbaturic acid (TBA) test as methods of analyzing the lipid oxidation state in subcutaneous lamb fat obtained from lambs fed either 15 IU or 300 IU supplemental α-tocopherol (vitamin E). The mPV and TBA analyses both demonstrated no significant difference ($P > 0.05$) in the lipid-oxidation state of the fat from four different loin sections ($n = 20$). Both mPV and TBA methods demonstrated virtually identical differences ($P < 0.05$) in the lipid-oxidation state of the fat derived from lambs fed two different levels of vitamin E. These results demonstrate the efficacy of employing mPV to monitor the lipid-oxidation in animal fat. The low temperatures inherent to the mPV method vs. the TBA method, along with mild extraction methods and speed of obtaining results, reduces the potential of causing spurious autoxidation or generating substances that are capable of interfering with the assay. Both mPV and TBA methods indicate that the level of dietary intake of vitamin E significantly affects the lipid oxidation-state of subcutaneous fat antemortem as well as seven days postmortem and, thus, the shelf life of lamb fat.

Introduction

Flavor is the trait responsible for consumer preferences for meat and meat products. Water-soluble compounds in the lean portion of muscle impart meat taste while the lipids contribute the flavors characteristic of lamb species (Horstein and Crowe, 1963). Lipid oxidation during prolonged storage or short-term exposure to high temperatures is often associated with "off flavors," "warmed over flavor," "rancid," and "stale" characteristics in mutton which result in product degradation and reduced case-life of an otherwise nutritious protein source.

One of the most important causes of meat food flavor deterioration is lipid oxidation, which affects fatty acids in general and polyunsaturated fatty acids in particular (Gray, 1978; Allen and Allen, 1981; and Fennema, 1993). Postmortem factors can influence lipid oxidation and decrease the shelf life of meat products due to the initiation of peroxidation (Vercellotti *et al.*, 1992). Oxidation of fatty acids in animal tissue starts to occur almost instantly after slaughter (Gray and Pearson, 1994).

Autoxidation of lipids is carried out by a free-radical chain reaction (Gray, 1978; Allen and Hamilton, 1983; Rojarho and Sofos, 1993). The initial step in this reaction is the generation of transitory hydroperoxides, which degrade into malonaldehyde (MA) and several other reactive compounds. Due to their unstable state, peroxides start their decomposition and form a series of secondary products, such as aldehydes, acids, and ketones, that produce undesirable rancid flavors (Shahidi, 1994).

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The most common chemical measurement of lipid oxidation in muscle foods is the thiobarbaturic acid (TBA) assay, based on the reaction of MA and TBA generating a TBA-MA complex with an absorbance maximum at 530 nm (Patton and Kurtz, 1951). Although widely employed, this method has limitations; the most significant being that both MA and TBA react with other substances present in meat and the extracts of meat. These substances produce aldehydic lipid oxidation products that can react with TBA. In addition, the nonspecificity of the assay is due to interfering compounds that react with TBA such as sugars, ascorbic acid, and nonenzymatic browning products (Decker *et al.*, 1998). Another potential drawback to the TBA method is that MA is often bound to proteins and the conditions for the optimal release of MA is often hard to determine. These forms vary from one sample to the next and require different hydrolytic conditions to release the MA. Also, it is difficult to release all of the MA from meat protein without employing strong acids and heat that adversely affect stability of the TBA-MA complex (Draper *et al.*, 1986).

A comparison of a modified peroxide value (mPV) and TBA (2-thiobarbituric acid) as methods for analyzing the lipid oxidation state of the lipids in the subcutaneous fat layer obtained from lambs fed two different levels of the lipid soluble antioxidant α-tocopherol (vitamin E) is reported here. These results demonstrate that mPV is a rapid, low-cost, simple, and reproducible method to monitor the lipid oxidation state in animal fat.

Materials and Methods

Comparison of TBA and mPV

For comparative statistical analysis of mPV and TBA methods, two samples of subcutaneous fat were removed from the right side of the loin and two samples from the left side of the loin. The lamb was obtained from The Ohio State University Meat Lab, Columbus, Ohio (Figure 1). The lamb had been fed an all-concentrate diet, offered *ad libitum*, formulated to provide 16% crude protein and 15 IU (NRC; Control) of supplemental vitamin E per kg of diet dry matter. It should be noted that lambs are normally fed 15 IU supplemental vitamin E per kg of diet dry matter and as such are considered control lambs. Carcass fabrication was conducted one-day post-slaughter.

Subcutaneous fat was removed, wrapped in a meat tray with polyvinyl-chloride oxygen-permeable film, and stored at 39.2 ± 3.6°F (4 ± 2°C) prior to analysis by both TBA and mPV.

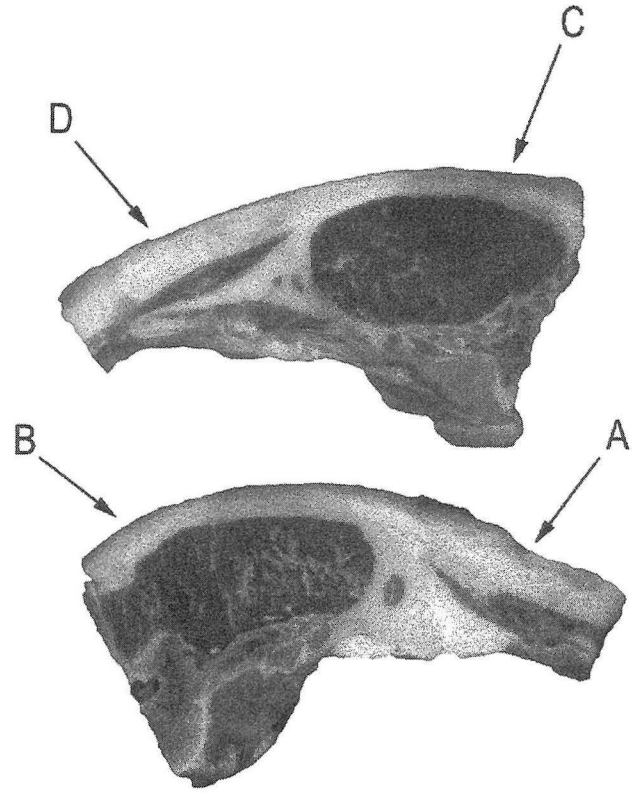

Figure 1. Sampling locations of subcutaneous fat from lamb loins. Right loin: A, B; Left loin: C, D.

Analysis of Lipid Oxidation State in Lambs Fed Two Vitamin E Supplements

A 2 (levels of vitamin E) x 4 (days of storage) factorial comparison of the lipid oxidation state in the subcutaneous loin fat in lambs fed two different levels of supplemental vitamin E was performed by employing both TBA and mPV. Twelve lambs were obtained from The Ohio State University's Ohio Agricultural Research and Development Center (OARDC) campus at Wooster, Ohio, where they had been fed an all-concentrate diet, offered *ad libitum*, formulated to provide 16% crude protein and either the control 15 IU supplemental vitamin E, recommend by National Research Council, or 300 IU supplemental vitamin E

per kg of diet dry matter, seven days before slaughter. Carcass fabrication was conducted seven days post-slaughter. Subcutaneous fat from the loin area was removed, wrapped in a Styrofoam meat tray with polyvinyl-chloride oxygen-permeable film, and stored at 39.2 ± 3.6°F (4 ± 2°C) from 1 to 11 days post-slaughter.

2-Thiobarbituric Acid (TBA) Assay

The degree of lipid oxidation in this experiment was determined by the TBA method described by Pensel (1990). Briefly, 5.0-grams of unrendered lamb fat were placed into a coded polyethylene stomacher bag. An additional empty stomacher bag was prepared as a blank. Then 50-mL of 39.2 ± 3.6°F (4 ± 2°C) 20% trichloroacetic acid (Fisher Scientific, Fair Lawn, N.J.) in 1.6% of m-phosphoric acid (Fisher Scientific, Fair Lawn, N.J.) solution was immediately added to each stomacher bag. Samples were blended in a Seward Laboratory blender (Tekmar Co., Cincinnati, Ohio) for 2 min. Then 50-mL of 39.2 ± 3.6°F (4 ± 2°C) distilled water were added to each bag for a second blending for 30 s. The slurry was filtered through a Whatman No. 1 filter. Five mL of freshly prepared 0.02 M 4, 6-dihydroxypyrimidine-2-thiol (Sigma Chemical Co., St. Louis, Mo.) was added to each tube and mixed for 4–5 s. Tubes were stored in the dark for 15 hours to develop the color. The color was measured by a Gilford Response UV-VIS Spectrophotometer (Ciba Corning Diagnostic Co., Oberlin, Ohio) at a wavelength of 530 nm.

A standard curve for the indirect determination of K was generated by reacting TBA with 1,1,3,3 - tetra-ethoxypropane (TEP) (Sigma Chemical Co., St. Louis, Mo.). A K value of 9.242 was obtained ($n = 20$, $R^2 = 0.996$). The TBA of each sample was determined employing the following formula:

$$\text{TBA } \mu g \text{ MA/g} = K \times O.D_{530nm}$$

Modified Peroxide Value (mPV)

The PV method of analysis was modified from the AOAC (1990) procedure. Briefly, a 50 g sample of unrendered subcutaneous loin fat each was ground in a Waring lab blender (Waring Products Division, Dynamic Cooperation of America, New Hartford, Conn.) for 20 to 30 s and extracted with 30 mL ice cold (3:2 v/v) acetic acid:chloroform. The extraction was vigorously swirled to distribute the sample and reagents. After the samples were dissolved in the acetic acid:chloroform mixture, 0.5 mL of saturated potassium iodine (KI) (83.2 g solid KI/40 mL H_2O) were added and mixed vigorously. Subsequently, 30 mL deionized water were added and the solution mixed thoroughly. Color of the upper aqueous layer ranged from pale yellow to bright yellow, with the lower organic layer remaining white. The mixture was allowed to stand for 5 to 10 min. at room temperature then titrated with 0.01 M $Na_2S_2O_3$ (Sigma Chemical, Fair Lawn, N.J.) gradually with vigorous shaking. During the titration, 0.5 mL of starch indicator (Starch 1% with chloroform 0.3%, Lab Chem., Inc., Pittsburgh, Pa.) was added. Color of the upper aqueous layer ranged from light purple to dark purple, and the lower organic layer remained white to gray. If the color of the lower organic layer remained yellow, the sample was vigorously swirled and allowed to stand for an additional 10 min. The end-point of titration was established when the color of the upper aqueous layer disappeared. The mPV was calculated employing the following formula:

$$mPV = \frac{(S)(N)(1000)}{W}$$

Where:

- mPV = Modified Peroxide Value (meq oxygen/kg fat)
- S = mL $Na_2S_2O_3$
- N = Normality of $Na_2S_2O_3$ (0.01 N)
- W = g of fat

Statistical Analysis

Statistical analysis was completed by statistical software SAS 7.0 for Windows® (SAS Institute, Inc., Cary, N.C.). Two-Way General Linear Models (GLM) were used to analyze the results.

Results and Discussion

Comparison of TBA and mPV Methodologies

The results reported in Table 1 indicate that both TBA and mPV methods detected no differences in the lipid oxidation state at four locations in the

subcutaneous loin fat ($P > 0.05$, $n = 20$). In addition, results also demonstrate the consistency and repeatability of both TBA and mPV. In addition, these data also indicate that lipids in the subcutaneous fat surrounding the loin from lambs fabricated within 24 hours of slaughter exhibit a very low oxidation state.

Table 1. Lipid Oxidation at Different Locations of Lamb Fat by TBA and mPV Methods.[1]

Locations N= 5/location	mPV (meq/kg)	TBA (μg/g)
A	2.48[a]	0.150[a]
B	2.36[a]	0.133[a]
C	2.04[a]	0.138[a]
D	1.96[a]	0.143[a]

[1] Means followed by the same superscripts within columns indicate that the values are not different ($P > 0.05$). Right loin: A, B; Left loin: C, D. TBA = 2-thiobarbaturic acid and mPV = modified peroxide value.

The comparative advantages of mPV in contrast to TBA as a method of analyzing the lipid oxidation state of lamb fat are summarized in Table 2. The ability of mPV to detect the oxidation of mono unsaturated fatty acids suggests that this method is more accurate in determining the oxidation state of all the fatty acids in the sample in contrast to the traditional TBA methodology. The potential for further autoxidation of the sample is minimized in the mPV method due to the use of non-acidic and ice-cold conditions during sample preparation and extraction. Additionally, the potential to compare the oxidation state in fat derived from different muscles is more appropriate with mPV than traditional TBA due to the fact that mPV detects only the degree of peroxide formation in the unconjugated fatty-acid fraction of the animal fat (Decker *et al.*, 1998). Finally, final color development for mPV is almost instantaneous while color development for the traditional TBA method requires at least 15 hours. This greatly shortens the "turn around" time for analysis of the lipid oxidation state of lamb fat by mPV versus the traditional TBA method.

Use of TBA and mPV to Determine Lipid Oxidation State of Fat from Lambs Fed Different Vitamin E Regimens

Both mPV and TBA methods demonstrate an increase in the lipid oxidation state in loin area subcutaneous fat obtained from lambs fed on both the control and 300 IU supplemental dietary vitamin E ($P < 0.05$). Both methods demonstrate similar lipid oxidation states of the fat from animals fed on both regimens until eleven days post slaughter. The greatest increase in 11-day lipid oxidation

Table 2. Comparative Analysis Between 2-Thiobarbaturic Acid (TBA) and the Modified Peroxide Value (mPV).

	mPV	TBA
Temperature of sample preparation (°C)	7 ± 2	25-100
Sample autoxidation potential	Low	High
Analysis time	2 h	18–24 h
Acidic conditions	No	Yes
Specificity for peroxides	Yes	No
Detection of oxidized mono and unsaturated fatty acids	Yes	No
Potential to compare oxidation between muscles with different fatty acid composition	Appropriate	Inappropriate

Table 3. Effects of Two Different Levels of Dietary Vitamin E on the Modified Peroxide Value (mPV) and 2-Thiobarbaturic Acid (TBA) Values of Lamb Fat During Storage.[1]

Day	mPV (meq/kg)		TBA (μg/g)	
Post Slaughter	Control	300 IU	Control	300 IU
1	1.90[a]	1.03[a]	0.167[a]	0.084[a]
7	2.08[ab]	1.09[a]	0.179[ab]	0.090[a]
9	2.33[b]	1.11[a]	0.193[b]	0.101[a]
11	3.50[c]	1.94[b]	0.254[c]	0.125[b]

[1] IU: International Unit. Total observation, N = 96. Means followed by different superscripts within columns indicate statistical differences are significantly different ($P < 0.05$)

was observed in the fat from lambs fed 15 IU (control animals) by both methods. There was no difference in the mPV or TBA of fat obtained from animals fed 300 IU vitamin E on days 1, 7, or 9, suggesting that high dietary vitamin E treatment retards the rate of lipid oxidation to a greater extent than control levels of dietary vitamin E treatment. In addition, lambs fed 15 IU vitamin E had higher initial mPV and TBA values than those fed 300 IU vitamin E (Table 3). Furthermore, mPVs significantly increased between day 9 and 11 ($P < 0.05$), indicating that increased dietary vitamin E treatment reduces the rate of lipid oxidation more than control dietary vitamin E intake, thus increasing the usable shelf life of lamb fat.

Implications

Previous research indicated that supplemental dietary vitamin E reduces lipid oxidation state of rendered fat from lamb meat. The rate of lipid oxidation is controlled by various antemortem factors. Composition of the diet is one of the most effective ways of inhibiting lipid oxidation in animal fat (Wulf *et al.*, 1995). In this study, lambs fed 300 IU of vitamin E apparently had lower mPVs than those fed 15 IU. These results suggest that higher levels of dietary vitamin E contribute to lower mPVs and less oxidation of subcutaneous lamb fat. In addition, increased levels and longer duration of dietary vitamin E contributed to a greater inhibition of lipid oxidation in the subcutaneous loin fat in lambs.

The mPV method reported here reproducibly analyzes the lipid oxidation state of fatty acids derived from animal tissue. The reaction time for the entire analysis is less than three hours. In contrast, the TBA method reported here is time consuming and incapable of detecting oxidized mono-unsaturated fatty acids.

The TBA method is primarily based on determining the concentration of a TBA-MA complex, a secondary oxidative product of fatty acid oxidation. However, MA is not present in all oxidized systems. In addition, the TBA method is not sensitive to the oxidation state of mono- or di-unsaturated fatty acid derivatives (Nawar, 1996). Gray (1978) reported no color development by TBA method for linoleate, a mono-unsaturated fatty acid, even though the peroxide value had already reached to 2,000 (Gray, 1978). Interaction of MA with available amino groups in meat components has been reported to affect the results of TBA analysis (Shahidi, 1994).

Lipid oxidation determinations are normally performed on animal fat samples more than seven days post slaughter. This is the first report of the lipid oxidation state determination on fresh (< 24 hours), unrendered, fabricated subcutaneous lamb fat. Both mPV and TBA yielded similar results, however; mPV is more rapid, simpler, more reproducible, costs less, and uses unrendered fat. These aspects of the mPV method could have a potentially great economic contribution for the meat industry.

Acknowledgements

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References

Allen, C. E. and E. Allen. 1981. Some lipid characteristics and interactions in muscle foods: a review. *Food Technol.* 35:253.

Allen, J. C. and R. J. Hamilton. 1983. *Rancidity in Foods.* Applied Science Publishers, London.

AOAC. 1990. *Official Methods of Analysis of the Association of Official Analytical Chemists.* Ch. 4, pp. 956. Association of Official Analytical Chemists, Inc. Arlington, Va.

Decker, E. A., Chan, W. K. M., and Faustman, C. 1998. TBA as an index of oxidative rancidity in muscle foods. Proc. 51st Ann. Recip. Meat Conf. p. 66. American Meat Science Assoc., Kansas City, Mo.

Draper, H. H., McGirr, L. G., and Hadley, M. 1986. The metabolism of malonaldehyde. *Lipids* 21(4):305.

Fennema, O. R. 1993. *Quimica de 10s alimentos.* Ed. Acribia, Zaragoza, Spain.

Gray, J. I. 1978. Measurement of lipid oxidation: A review. *J. Am. Oil Chem.* Soc. 55:539–546.

Gray, J. I. and Pearson, A. M. 1994. Lipid-derived off-flavor in meat-formation and inhibition. In: *Flavor of Meat and Meat Products.* 1st Ed. pp. 117-139. Chapman & Hall, London, U.K.

Hornstein, I. and Crowe, P. F. 1963. Meat flavors: Lambs. *J. Agric. Food Chem.* 11:147.

Nawar, W. W. 1996. Lipids. In: *Food Chemistry.* 3rd Ed. pp. 226, 255–273. Marcel Dekker, Inc., New York, N.Y.

Patton, S. and Kurtz, G. W. 1951. 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation. *J. Dairy Sci.* 34(6):699.

Pensel, N. A. 1990. Influence of experimental conditions on porcine muscle and its effect on oxidation. Masters Thesis. Department of Animal Sciences, The Ohio State University, Columbus, Ohio.

Raharjo, S. and Sofos, J. N. 1993. Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. *Meat Sci.* 35:145.

SAS. 1998. *SAS User's Guide: Statistics and statistical analysis system, version 7.* SAS Institute, Inc., Cary, N.C.

Shahidi, F. 1994. Assessment of lipid oxidation and off-flavor development in meat and meat products. In: *Flavor of Meat and Meat Products.* pp. 247-266. Chapman & Hall, London, U.K.

Vercellotti, J. R., St. Angelo, A. J., and Spanier, A. M. 1992. Lipid oxidation in foods: An overview. In: *Lipid Oxidation in Food.* pp. 1-11. American Chemical Society, Washington, D.C.

Witte, V. C., Krause, G. F., and Bailey, M. E. 1970. A new extraction method for determining 2-Thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* 35:582–585.

Wulf, D. M., Morgan, J. B., Sanders, S. K., Tatum, J. D., Smith, G. C., and Williams, S. 1995. Effects of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts. *J. Anim. Sci.* 73:399–405.

Impact of the Hydrodyne® Process on Tenderness of Pork

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Abstract

Paired boneless pork loins were obtained from 76 market hogs to evaluate the tenderness, meat quality characteristics, sensory attributes, and microbial content of pork loins exposed to the Hydrodyne® Process (HP). The Hydrodyne® Process uses a small explosion to generate a shock wave in water. The resulting shock wave, when administered to meat with similar acoustic properties as water, passes through the meat and creates substantial damage to muscle structure, resulting in improved tenderness. Research on the impact of this technology on pork is limited, and this study was designed to evaluate the impact of the Hydrodyne® Process on tenderness in pork.

Administration of the Hydrodyne® Process resulted in a 17% improvement in tenderness over the nontreated control (C) loin when comparing Warner-Bratzler shear-force values. The effect of the HP on tenderness was consistent when cooked to two end-point temperatures, and no differences in cooking loss were observed when comparing HP and C loins at both end-point temperatures. No differences between HP and C were observed for color score, firmness score, Hunter L color, Minolta reflectance, or drip loss on uncooked samples. HP loins were found to have significantly lower marbling scores and intra-muscular fat contents than the paired C loin from the same pig. A subset of 16 paired loins was randomly selected and tested for sensory attributes. The loin samples used in the taste panel did not exhibit a significant improvement in Warner-Bratzler shear tenderness as a result of the hydrodyne treatment, which is in contrast to the improvement observed in the analysis of the entire set of loins. Sensory panelists were also unable to differentiate treatment differences between HP and C loins for both initial or sustained tenderness scores. Sensory evaluation on the subset of 16 paired loins also showed no differences in HP or C loins for pork flavor, off-flavor, cohesiveness, or number of chews prior to swallowing, but HP loins had a significantly higher juiciness score and more cooking loss than C loins. Microbial analysis on the subset of 16 loins showed no differences in coliform bacteria counts or aerobic plate counts for HP or C loins and no detectable levels of *E. coli* bacteria in either HP or C loins.

Introduction

The development and implementation of carcass merit payment systems in the U.S. swine industry has resulted in dramatic changes in the lean composition of the swine produced over the last five to 10 years. Changes in genetics, feeding programs, and management strategies have all played a role in producing leaner, more efficient swine. Unfortunately, the demand for increased lean has resulted in undesirable changes in muscle quality traits including reduced intra-muscular fat content, lower ultimate pH, and poor water-holding capacity. Changes in these and other muscle quality traits have been shown to be related to the tenderness and overall acceptability of pork products when

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compared by trained sensory panels and in consumer preference trials (NPPC 1995, Terminal Line Program Results). Because tenderness is an important criteria in determining consumer acceptability of pork, exploration of new technologies to improve tenderness of pork should be addressed.

The Hydrodyne® Process, a relatively new procedure designed to improve tenderness of meat, was reported by Solomon and Long, 1995. They reported the Hydrodyne® Process uses a small amount of explosive to generate a shock wave in water, creating a pressure front of 10,000 psi at the point of contact with an object. The shock wave occurs in fractions of a millisecond and passes through objects in the water that are acoustic matches with water. Since meat is a good acoustic match with water, the HP process has an effect on meat. As the shock wave passes through the meat, a compression occurs in advance of the wave front and negative pressure occurs after its passage. If the shock pressure is large enough, a tenderizing effect (Solomon *et al.*, 1995) brought about by destruction of muscle structure results. The Hydrodyne® Process has been shown to significantly increase tenderness in beef (Solomon and Long, 1995) and sheep (Solomon *et al.*, 1995) muscles and has been viewed as a revolutionary approach to improve meat tenderness requiring less space, energy, and labor costs (Solomon *et al.*, 1995) than alternative approaches. Research efforts to characterize the impact of the Hydrodyne® Process on pork muscle are important as the U.S. industry attempts to improve the eating quality and consumer acceptability of pork and seeks to increase both domestic and foreign demand for U.S. pork products.

Objectives

The purposes of this study were to:

- Determine the effect of the Hydrodyne® Process on tenderness, color attributes, drip loss, and cooking loss of pork longissimus muscle.
- Determine the effect of the Hydrodyne® Process on eating-quality characteristics of the longissimus muscle when compared by a trained sensory panel.
- Determine the effect of the Hydrodyne® Process on microbial load or contamination of pork.

Procedures

Animals

Pork muscle used in this study originated from 76 market animals, randomly selected from the 1996 Iowa State University, Livestock Producers Assistance Program SEW Test conducted at the Northeast Iowa Swine Improvement Association swine-testing station in New Hampton, Iowa. Following slaughter and carcass data collection at Hormel Foods™, Austin, Minnesota, paired loins (*longissimus* muscle, 10th rib through sirloin end) from each pig were de-boned, trimmed of external fat, vacuum packaged in an oxygen impermeable bag, and frozen.

Hydrodyne® Process

Frozen loins were transported to the USDA Beltsville Agricultural Experiment Station where the Hydrodyne® Process was conducted. One loin from each pig was assigned to either a control (C) (n=76) or HP (n=76) treatment protocol. All loins were thawed prior to administration of the HP. The HP process was conducted using standard protocols outlined by Solomon and Long (1995). The HP was conducted using groups of individually vacuum-packaged loins placed into a single, large rubber bag. A subset (n =16 paired loins) of the C and HP loins was randomly selected to be used for sensory evaluation and microbial characterization. Loins designated for sensory and microbial evaluation were individually treated in the HP protocol to avoid any possible cross contamination of samples.

Muscle Quality Procedures

Upon completion of the HP procedure, all loins (n = 152) were refrozen and transported to The Ohio State University. Upon arrival at Ohio State, loins were thawed and samples collected for further testing. Muscle-quality tests conducted included subjective, visual assessment of color, marbling, and firmness; objective, Minolta reflectance and color scores; drip-loss estimation of uncooked samples; and total lipid extraction.

Tenderness Evaluation

Two loin samples from each C and HP-treated loin (total of four samples per pig) were cut to a standard thickness of one inch. One loin sample from each treatment (C or HP) was assigned to one of two designated cooking times (11 or 16 minutes) to determine the effect of internal temperature on Warner-Bratzler shear-force values. All loin samples were cooked in an impingement oven set at 375°F, with the samples placed on an adjustable speed conveyor to standardize cooking time. Loin samples were weighed prior to and following cooking to determine cooking loss. Internal temperature on the cooked loin samples was recorded for use in data analysis.

Warner-Bratzler shear-force values were recorded on cooked loin samples to determine the effect of treatment and cooking time on the tenderness of the loin samples. Shear-force values were collected from six individual core samples per cooked loin, with the average of the six values used in data analysis for measurement of tenderness by the Warner-Bratzler shear method.

Sensory Evaluation

The subset of paired loin samples from 16 pigs (n = 32 total loins) was used to evaluate sensory attributes in a blind taste test by a trained sensory panel. The taste panel consisted of eight individuals who were randomly assigned to taste the loin samples from two pigs. In the experimental design, four individual tasting sessions were held, and each panelist tasted C and HP loin samples from two specific pigs in replicate. In total, 128 observations (16 pigs *4 samples/pig * 2 replications/pig) were recorded and used in the analysis. The four observations per pig consisted of two replicates of C and HP loins. Sensory traits evaluated included pork flavor, off-flavor, initial tenderness, sustained tenderness, juiciness, cohesiveness, and the number of chews necessary to consume the sample.

Microbial Characterization

Paired loin samples representing the subset of 16 pigs (n = 32 total) were collected using sterilized equipment, bagged, and sent to an analytical lab for testing. Samples were tested for coliform bacteria (mpn/g), *E. coli* bacteria (mpn/g), and aerobic plate counts (25ºC, three days). Microbial counts were converted to logarithmic function for analysis.

Statistical Analysis

The data collected in the study were analyzed using statistical procedures outlined in SAS (1994). All data were analyzed using mixed model procedures with pig considered to be a random effect in the testing of differences among treatments (C or HP). Shear-force and cooking-loss data were analyzed using a second fixed effect of cooking time (11 or 16 minutes) and the interaction of treatment and cooking time. Sensory evaluation data were analyzed using panelist as a second random variable when testing treatment differences. The microbial characterization data were converted to a $\log_{10}$ scale and analyzed using a model with random effects of pig in the analysis of treatment differences.

Results

Table 1 summarizes the results of muscle quality traits measured on the HP and C loin muscle samples. No significant differences were observed between the HP- and C-treated loins for visual assessments by color score or machine-measured light reflectance (Minolta) and color (Hunter L and Hunter b*), indicating that the HP process did not affect the overall appearance of pork loins. Significant differences were found for Hunter a*, indicating that HP-treated loins had reflectance values that were less red than C loins. Loins treated with the HP protocol also had significantly lower marbling scores and intra-muscular fat content than C loins from the same pig. This finding is not easily explained from a biological or procedural standpoint and is an area that needs to be investigated in the future. The results of this study indicate no negative effects on commonly measured muscle- quality traits of pork, including color, firmness, and water-holding capacity when treated by the HP protocol. The observed drip loss values were extremely low for both C (0.81%) and HP (0.73%) loins.

The effect of HP on tenderness and cooking loss of the loin muscle is described in Table 2. Loins treated by the HP had shear-force values of 2.69 kg compared to 3.24 kg for the control, resulting in a 17% improvement in tenderness. Tenderness

Table 1. Least Squares Means and Standard Errors for *Longissimus* (Loin) Muscle Quality Traits for Hydrodyne® Treated and Control Samples of Loin from the Same Pig.

		Treatment		
Trait[a]	N	Control	Hydrodyne®	Significance[b]
Color	76	2.84 ± 0.04	2.90 ± 0.04	NS
Marbling	76	3.02 ± 0.04	2.86 ± 0.04	+
Firmness	76	2.89 ± 0.04	2.74 ± 0.04	NS
Intra-muscular fat, %	69	2.69 ± 0.06	2.48 ± 0.06	*
Minolta reflectance	76	25.91 ± 0.24	26.00 ± 0.24	NS
Hunter L color	76	50.79 ± 0.23	50.88 ± 0.24	NS
Hunter a*	76	14.40 ± 0.09	14.85 ± 0.09	**
Hunter b*	76	7.11 ± 0.06	7.15 ± 0.06	NS
Drip loss, %	76	0.81 ± 0.11	0.73 ± 0.11	NS

[a] Color, 1–5 scale, 1 = pale and 5 = dark; Marbling, 1–5 scale, 1 = devoid and 5 = excess; Firmness, 1–5 scale, 1 = soft and wet and 5 = firm and dry; Minolta reflectance, 0 = no reflectance, 100 = total reflectance; Hunter L color, 0 = black and 100 = white; Hunter a*, red/green hue, higher number = more red; Hunter b*, blue/yellow, higher number = more yellow; Drip loss = percentage of exudate from noncooked sample.

[b] Significance level: NS = no significant difference between Hydrodyne® and control: + $P < 0.10$, * $P < 0.05$, ** $P < 0.01$.

Table 2. Least Squares Means and Standard Errors of *Longissimus* (loin) Muscle Tenderness and Cooking Loss Traits for Hydrodyne® Treated and Control Samples of Loin Cooked to Two Endpoint Times.

		Treatment			
Trait[a]	N	Control	Hydrodyne®	Sig[b]	% Difference
Warner-Bratzler shear					
Overall	152	3.24 ± 0.04	2.69 ± 0.04	***	17.0
11 minute cooking	76	3.19 ± 0.05	2.71 ± 0.05	***	15.0
16 minute cooking	76	3.30 ± 0.05	2.68 ± 0.05	***	18.8
		Cooking Time			
		11 minutes	16 minutes	Sig[b]	% Difference
Cooking Loss, %					
Overall	152	26.39 ± 0.27	34.04 ± 0.27	***	22.5
Control	76	26.51 ± 0.37	34.22 ± 0.37	***	22.5
Hydrodyne	76	26.25 ± 0.37	33.85 ± 0.37	***	22.5

[a] Warner-Bratzler shear force = kg of pressure required to shear a standard core of muscle; Cooking Loss, % = ((raw weight-cooked weight)/raw weight) * 100.

[b] Statistical significance level for means within a row: *** $P < 0.001$.

differences between HP and C were consistent across cooking time (11 or 16 min.) with the HP-treated loins 15% and 18% more tender than controls at 11 and 16 minutes, respectively. The effect of HP on cooking loss was not significant in the study, indicating that cooking-loss differences were not observed when comparing HP and C loins. Time of cooking, as expected, had a significant effect on cooking loss with loins cooked for 16 minutes having 22.5% more cooking loss for both HP and C loins cooked for 11 minutes. Data analysis results also show no significant interaction exists between Treatment (C or HP) and Cooking Time (11 or 16 min.), which means the impact of HP on tenderness was consistent across cooking times. It should be noted that the loins utilized in the study were frozen and thawed prior to HP treatment and collection of shear-force data, which is different than other experiments evaluating HP on other species. The impact of the HP protocol on fresh, never frozen, pork cannot be determined in this study. However, the results on frozen pork indicate that the HP protocol is an effective method of improving tenderness as measured by Warner-Bratzler shear force, an objective method of measuring meat tenderness.

Sensory evaluation scores are presented in Table 3. Significant differences were found between HP and C treatments for juiciness, with Hydrodyne loins having lower juiciness scores (4.87 vs. 5.20 units) than the Control. However, no differences were reported for initial tenderness, sustained tenderness, cohesiveness, or the number of chews necessary to consume the sample. Warner-Bratzler shear-force values on the subset of 16 paired loins were not different, which explains the failure to detect sensory differences in initial and sustained tenderness of the HP and C loins. Differences in pork-flavor and off-flavor approached significance ($P < 0.10$) with the HP-treated loins having slightly less off-flavor and higher pork-flavor scores than the control loin samples. The sensory evaluation findings suggest that the HP protocol had no major effect on sensory characteristics other than juiciness. Conclusions on the effect of HP treatment upon sensory evaluation of tenderness can not be clearly made because the subset of loins designated for sensory evaluation did not respond in the same manner as the entire set of 76 paired loins where a 17% improvement in Warner-Bratzler shear force was observed. The reason a random set of 16 loins, chosen from a set of 76 loins, did not show a treatment effect may be due to chance and a small number of observations.

The results of the microbial tests conducted on the paired loin samples (n = 16 pigs) used in the sensory evaluation are presented in Table 4. Plate counts were converted to a logarithmic scale in the analysis. No significant differences between C and HP treatments were observed for coliform bacteria or aerobic plate count. No detectable levels of *E. coli* were observed in any samples.

Summary

The results of the study indicate that use of the HP protocol is an effective method of improving tenderness of pork-loin muscle when measured by Warner-Bratzler shear force. However, the improvement in Warner-Bratzler shear force (17%) was not detectable in the subset of loins utilized for trained sensory evaluation, resulting in no observable differences in taste panel scores for initial or sustained tenderness of pork loin.

The observed improvement in Warner-Bratzler shear force resulting from the HP treatment was determined to have no detrimental effects on visual or objective measures of color, loin water-holding capacity measured by visual firmness/wetness scores, drip loss and cooking loss, or microbial growth and contamination. Sensory evaluation showed HP loins were less juicy than C loins, but no differences were found for pork flavor, off-flavor, initial or sustained tenderness, or cohesiveness of the loin. Pork loins treated with HP had significantly lower marbling scores and intra-muscular fat percentage than C loins and a higher cooking loss. This may explain why the sensory panel observed HP-treated loins were less juicy.

The overall importance of this research for the swine industry is that the Hydrodyne® Process, a process that requires little space, time, or energy, appears to improve tenderness of the pork loin without having major detrimental affects on muscle quality traits, sensory characteristics, or the safety and wholesomeness of pork. The observed differences in Warner-Bratzler shear force on the pork loin, one of the most tender cuts of pork, warrants further research on less tender cuts of

Table 3. Least Squares Means and Standard Errors for Sensory Panel Attributes of Hydrodyne® Treated and Control Loin Samples.

		Treatment		
Trait[a]	N	Control	Hydrodyne®	Sig[b]
Pork-flavor	16	5.61 ± 0.18	6.06 ± 0.18	+
Off-flavor	16	1.18 ± 0.05	1.04 ± 0.05	+
Initial tenderness	16	5.73 ± 0.15	5.79 ± 0.15	NS
Sustained tenderness	16	6.17 ± 0.16	5.96 ± 0.16	NS
Warner-Bratzler Shear	16	2.77 ± 0.05	2.74 ± 0.04	NS
Cook loss, %	16	29.69 ± 0.59	31.65 ± 0.43	*
Juiciness	16	5.20 ± 0.17	4.87 ± 0.17	*
Cohesiveness	16	5.23 ± 0.15	5.14 ± 0.15	NS
Number of Chews	16	17.59 ± 0.31	17.42 ± 0.31	NS

[a] Pork-flavor, 1-10 scale, 1 = none & 10 = intense; Off-flavor, 1–10 scale, 1 = none and 10 = intense; Initial tenderness, 1-10 scale, 1 = very tough & 10 = very tender; Sustained tenderness, 1–10 scale, 1 = very tough and 10 = very tender; Warner-Bratzler shear = kg of force to shear the sample, Cook loss = moisture loss during cooking, Juiciness, 1-10 scale, 1 = very dry and 10 = very juicy; Cohesiveness, 1–10 scale, 1 = very chewy and 10 = non-chewy ; Number of chews = number of chews necessary to completely eat the sample.

[b] Statistical significance level: NS = no significant difference between HP and C loins, + $P < 0.10$, * $P < 0.05$.

Table 4. Least Squares Means for Microbial Characteristics of Hydrodyne® Treated and Control Pork Loins.

		Treatment		
Trait[a]	N	Control	Hydrodyne®	Sig[b]
Coliform Bacteria Count	16	0.80 ± 0.26	1.40 ± 0.26	NS
E. coli Bacteria Count	16	ND	ND	
Aerobic Plate Count	16	4.38 ± 0.17	4.50 ± 0.17	NS

[a] Coliform Bacteria Count: level indicated is $\log^{10}$ the described plate count; *E. coli* Bacteria, Not detectable; Aerobic Plate Count, (25ºC for 3 days), level indicated is $\log^{10}$ the described plate count.

[b] Statistical significance level: NS = no significant difference.

pork and necessitates evaluation of the feasibility of using the Hydrodyne® Process in the packing industry. The use of the Hydrodyne® Process may be a tool that can be effectively utilized by the pork industry to improve overall tenderness of pork products produced, but it should not be considered a short cut to improving tenderness problems associated with major genes, genetic types, nutrition programs, or management styles that contribute to tough pork. Techniques such as the Hydrodyne® Process must be evaluated for their impact and place in the industry effort to improve pork products along the entire pork chain.

References

National Pork Producers Council. 1995. Genetic Evaluation: Terminal Line Program Results.

Solomon, M. B. and Long, J. B. 1995. The Hydrodyne® Process for tenderizing meat. *J. Anim. Sci.* Lopes, 73 (Suppl. 1): 159. Abstract.

Solomon, M. B., Long, J. B., Eastridge, J. S., and Carpenter, C. E. 1995. Tenderizing Callipyge lamb with the Hydrodyne® Process. 41st International Congress of Meat Science and Technology. 1995. San Antonio, Texas. pp 622–623.

Effects of Storage and NaOH Treatment on the Quality of Cured, Cooked Hams from Hampshire Hogs

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Abstract

In order to evaluate processing quality effects of the Napole gene (RN^-, rn^+) and the feasibility of improving the functionality of pork from hogs possessing the unfavorable dominant allele (RN^-), paired hams from 40 hogs (selected from a Hampshire progeny test conducted at the Western Illinois Test Station) were obtained. One ham of each pair was stored non-frozen and the other frozen. Each ham was divided in half after storage and two processing scenarios were applied to each of the ham halves, one of which was intended to improve the functional quality of pork over a conventional processing method. The addition of 0.125% sodium hydroxide (NaOH) to a conventional injection solution increased cooked pH, cooking yields, and darkened product color over the control. No differences in purge quantities were found between the two treatments. These results showed that some of the detrimental effects of the Napole gene can be mitigated by altering processing techniques, but the scenarios tested here also benefited the normal (rn^+) hams, maintaining an advantage to not having the gene. A sensory panel was also performed on these hams, and NaOH treatment was found to increase both tenderness and juiciness. A second, smaller study was also performed and supported the results of the first study.

Introduction

The Napole Gene is reported to be a major dominant gene influencing meat quality, particularly drip loss and cooking loss during processing of pork. The highest frequency of the gene is reported to be in the Hampshire breed.

Results of University of Illinois research indicate a significant economic loss to packer-processors due to increased drip and cooking loss of pork from pigs with the Napole gene. Some packers are requesting that commercial producers restrict their use of Hampshire boars, and some breeding companies are choosing to remove Hampshire genetics from their genetic lines. However, the superior leanness and carcass lean yield of the Hampshire is an asset the U.S. pork industry should not discard. In addition, the Hampshire breed is widely recognized as a genetic resource for the production of high-quality pork.

The Hampshire breed would benefit from increased understanding of the physiological mechanisms underlying the problem and possible alternate processing strategies that may mitigate the problem.

Materials and Methods

Paired hams, from hogs selected from a Hampshire progeny test conducted at the Western Illinois Test Station, were obtained from the Farmland plant in Monmouth, Illinois. Selection of hogs was based upon glycolytic potential (GP). Hogs with GP values of 180 and below were considered low GP, and hogs with GP values of 250 and above

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were considered high GP. Paired hams from 40 hogs were obtained for processing and analysis, depending upon the GP values and characterization of hogs. Only 33 pairs were included in the study due to product loss, loss of identification, or lack of GP value. Obtaining paired hams allowed for a blocked comparison of two storage conditions and two processing scenarios. One ham of each pair was stored non-frozen (30°F) and one was stored frozen (-10°F). Hams were divided in half, which allowed for two processing scenarios, one of which was intended to improve the functional quality of this pork over a second, conventional processing method.

After surface fat and connective tissue were removed, each ham muscle was vacuum packaged and stored for approximately one month unfrozen or frozen to compare the storage treatments. After storage, each ham was ground through a three-quarter-inch plate, and pH was determined. Nonmeat ingredients were mixed with one ham of each pair, to simulate the levels of ingredients conventionally added to cured hams in the United States (e.g., 25% injection above green ham weight), with the identity of each ham mixture being maintained. To the other ham of each pair, a solution designed to maximize the pH and water-holding capacity of meat was added to the ground meat.

The solutions consisted of the following:

Conventional solution:

28% water, 2% sodium chloride, 0.5% sodium tripolyphosphate/hexametaphosphate mixture, 0.020% sodium nitrite, and 0.055% sodium erythorbate.

Test (NaOH) solution:

28% water, 2% sodium chloride, 0.5% sodium tripolyphosphate/hexametaphosphate mixture, 0.020% sodium nitrite, 0.055% sodium erythorbate, and 0.125% sodium hydroxide.

There was one difference in the curing solutions between storage treatments. Sodium nitrite and erythorbate were only present in the curing solutions of those hams that were frozen. Therefore, no nitrite or erythorbate ($-NO_2$) was a confounding variable with unfrozen hams in this project.

After mixing, samples of each ham/treatment were stuffed into pre-smoked, fibrous casings, fully cooked to an internal temperature of 155°F, chilled, and vacuum packaged. After cooking, pH, cooking yields, cured internal color (Minolta L*, a*, and b*), and package purge were determined for each ham/treatment. Vacuum-packaged ham slices were stored for four to six weeks, to determine the content of accumulated purge in packages over time.

A sensory panel study was also conducted on the frozen processed hams from both chemical treatments to evaluate sensory effects of ham from RN^- pigs and the effects of using NaOH to improve RN^- meat quality. Half of the ham samples were controls and the other half were samples with 0.125% NaOH. Each of the eight semi-trained members of the taste panel was served 66 samples of ham over four days. The panelists evaluated the sensory attributes of juiciness, tenderness, ham flavor intensity, and off-flavor intensity. An eight-number scoring scale was used, with one being the lowest score and eight being the highest.

A second study (phase 2) was also conducted on eight paired hams obtained from Herman Falter Packing Co., Columbus, Ohio. Glycolytic potential was measured at The Ohio State University; however, animals were not able to be classified in low and high GP groups due to the low number of hams selected for the study. Hams were processed in the same methods as the first study except all hams were processed at the same time, under the same conditions, and all hams contained nitrite and erythorbate. Package purge and pH were also measured after storage treatment.

Results and Discussion

Hams from high GP animals were found to have decreased pH, cooking yield, and Minolta L* (lightness) value compared with low GP animals. Hams from RN^- animals also had increased Minolta b* (yellowness) value and tenderness (conventional solution only). No differences in Minolta a* (redness) value, cured-product package purge, juiciness, ham-flavor intensity, and off-flavor intensity were found between GP groups.

The decrease in Minolta L* value of hams from RN^- pigs was in contrast to previous research where RN^- pigs were found to possess paler meat color (Enfalt *et al.*, 1994; LeRoy *et al.*, 1996; Lundstrom *et al.*, 1996). However, most of this research studied fresh meat and Longissimus dorsi

muscle. Ellis *et al.* (1997) reported lower Minolta L* values in the Gluteus Medius of the ham from RN^- compared to rn^+ animals, supporting the results from this study.

Both the freezing treatment and NaOH treatment were found to significantly increase the pH, cooking yield, and decrease Minolta L* and b* values of cooked hams (Table 1). The significant increase in pH of hams treated with NaOH was expected, as NaOH is a strong base. The significant increase in cooking yield from NaOH addition supported previous research where higher cooking yields were observed with the addition of NaOH (Knipe *et al.*, 1988; Anjaneyulu *et al.*, 1990). Phase 2 results (Table 2) supported the increased cooking yield results of phase 1. The increase in cooking yield from both the frozen and NaOH treatments was found to be greater in hams from RN^- animals than rn^+ animals (Figure 1 and 2). This is important if more comparable cooking yields between GP groups are desired. Sutton (1997) supported this research by reporting comparable cooking yields of pork from RN^- and rn^+ animals, in buffered protein gels.

Table 1. Phase 1 Storage and Chemical Treatment Effects on Cured, Cooked Ham Quality Attributes at Mean Glycolytic Potential[1].

	Non-Frozen (-NO_2)		Frozen		
Variable	**Control**	**NaOH**	**Control**	**NaOH**	**SE**
Cooked pH[2]	5.77[a]	6.13[b]	5.92[c]	6.29[d]	0.02
Yield	88.22[a]	90.76[b]	90.59[b]	92.61[c]	0.39
L*	64.81[a]	63.57[b]	64.07[c]	62.07[d]	0.22
a*	10.11[a]	10.35[a]	14.81[b]	15.01[b]	0.19
b*	8.02[a]	7.81[a]	6.80[b]	5.95[c]	0.09
Package Purge[3]	2.78[a]	3.03[a]	1.21[b]	1.37[b]	0.14
Package Purge[4]	6.87[a]	7.16[a]	3.96[b]	4.18[b]	0.29

[1] Data reported at mean glycolytic potential (225.05 µmol/g) across all hams (n = 132).
[2] Means followed by different letters in the same row are different, $P < 0.05$.
[3] Package purge measured by pouring out purge.
[4] Package purge measured by pouring out purge and patting dry each ham slice.

Table 2. Phase 2 Storage and Chemical Treatment Effects on Cured, Cooked Ham Quality Attributes at Mean Glycolytic Potential[1].

	Non-Frozen		Frozen		
Variable	**Control**	**NaOH**	**Control**	**NaOH**	**SE**
Cooked pH[2]	6.21[a]	6.57[b]	6.27[a]	6.57[b]	0.04
Yield	92.72[a]	94.03[b]	93.60[b]	95.27[c]	0.22
L*	61.89[a]	61.57[a]	61.65[a]	60.31[b]	0.56
a*	15.73[a]	15.57[a]	15.82[a]	16.06[a]	0.24
b*	5.93[acd]	5.27[b]	6.13[c]	5.72[d]	0.09
Package Purge[3]	1.38[a]	1.39[a]	1.37[a]	1.08[a]	0.19
Package Purge[4]	2.42[a]	2.79[a]	2.41[a]	1.85[a]	0.36

[1] Data reported at mean glycolytic potential (92.44 µmol/g) across all hams (n = 32).
[2] Means followed by different letters in the same row are different, $P < 0.05$.
[3] Package purge measured by pouring out purge.
[4] Package purge measured by pouring out purge and patting dry each ham slice.

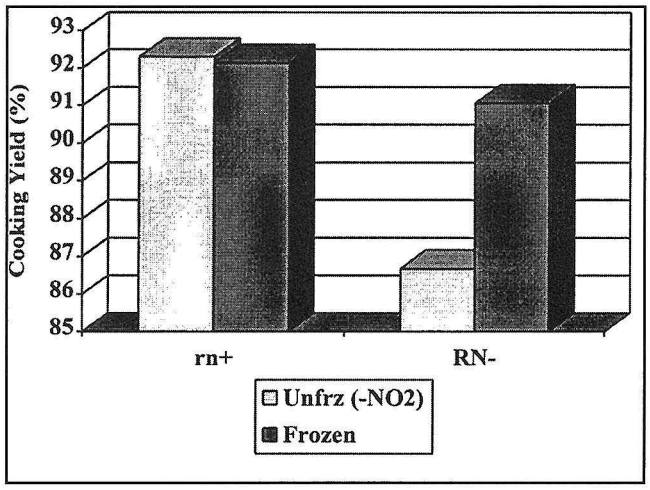

Figure 1. Two-way interaction (storage*genotype) on the cooking yield of cured, cooked ham.

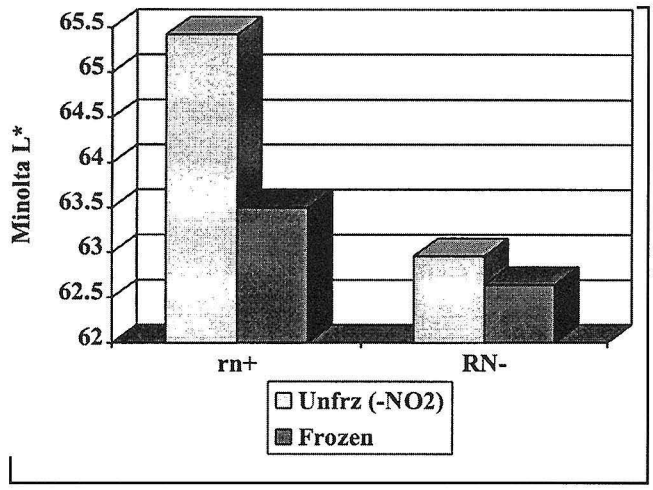

Figure 3. Two-way interaction (storage*genotype) on Minolta L* of cured, cooked ham.

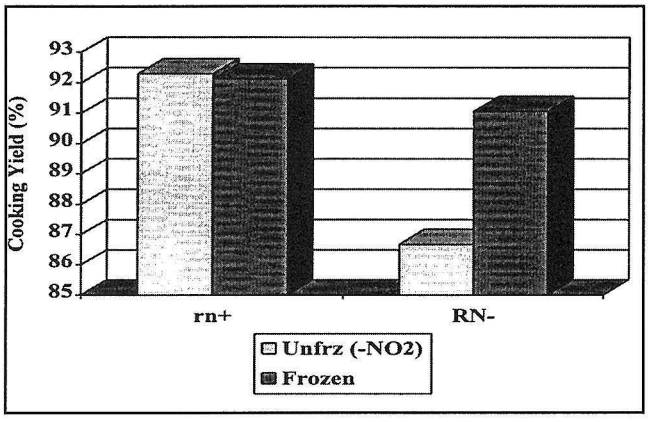

Figure 2. Two-way interaction (chemical treatment * genotype) on the cooking yield of cured, cooked ham.

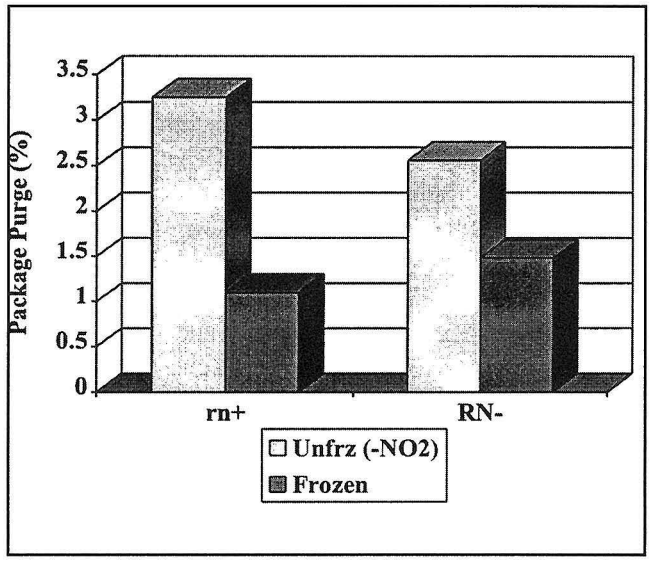

Figure 4. Two-way interaction (storage*genotype) on package purge (pour) of cured, cooked ham.

The decrease in Minolta L* from frozen storage was found to be greater in the lighter colored hams of rn^+ animals (Figure 3). The lowest Minolta L* values were found in those hams treated with frozen storage and NaOH (Table 2). Frozen hams were also found to possess significantly less package purge after raw and cured product storage. The decrease in cured-product package purge from frozen storage was found to be greater in the hams of rn^+ animals (Figure 4).

No significant differences were seen between low and high GP groups for any of the four sensory characteristics. However, high GP animals showed trends towards higher juiciness and tenderness. Sensory results of NaOH-treated hams found significantly more tenderness and juiciness than controls (Table 3). Increased juiciness was also reported in NaOH-treated beef rolls, compared to controls, in previous research (Moiseev and Cornforth, 1997).

For those hams processed with the conventional solution, a significant increase in tenderness was observed for hams from RN^- animals (Figure 5). However, when NaOH was added, an increased and more consistent tenderness was observed across GP. No significant difference was found between chemical treatments for ham-flavor intensity and off-flavor intensity, indicating panelists were unable to detect any off-flavor or loss of ham-flavor which may accompany the addition of NaOH.

Table 3. Chemical Treatment Effects on Sensory Attributes of Cured, Cooked Ham.

Sensory Attributes[1,2]	Control	NaOH	SE
Juiciness	5.21[a]	5.48[b]	0.11
Tenderness	5.46[a]	5.70[b]	0.11
Ham-Flavor Intensity	5.53[a]	5.41[a]	0.10
Off-Flavor Intensity	3.21[a]	3.15[a]	0.15

[1] Means followed by different letters in the same row are significantly different, $P < 0.05$.
[2] Juiciness, 8 = extremely juicy, 1 = extremely dry; Tenderness, 8 = extremely tender, 1 = extremely tough; Ham-Flavor Intensity, 8 = extremely intense, 1 = extremely bland; Off-Flavor Intensity, 8 = extremely intense, 1 = extremely bland.

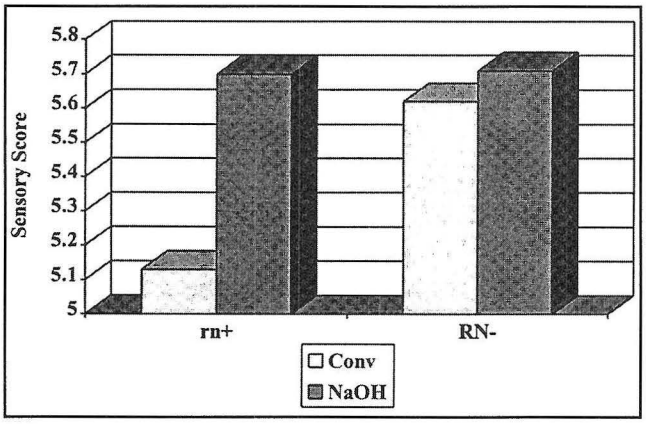

Figure 5. Two-way interaction (chemical treatment * genotype) on the tenderness of cooked, cured ham (rn^+ = normal ham and RN^- = ham with Napole gene).

Conclusions

Hams from RN^- pigs showed advantages of darker cured color and increased tenderness. The increased pH, cooking yields, and decreased lightness observed after treatments of NaOH and frozen storage indicate that both are effective for improving the quality of meat from RN^- animals, and to a smaller degree, rn^+ animals. The tenderness and juiciness preferences reported by taste panelists for NaOH-treated hams supplement the improved cooking yields and color. The addition to hams of frozen storage and treatment with 0.125% NaOH would be very beneficial to pork processors. Not only do these treatments help to decrease the difference in the quality of meat between RN^- and rn^+ pigs, they also slightly improve the quality of meat from rn^+ pigs.

References

Anjaneyulu, A. S. R., Sharma, N., and Kondaiah, N. 1990. Specific effect of phosphate on the functional properties and yield of buffalo meat patties. *Food Chemistry* 36: 149-154.

Ellis, M., McKeith, F. K., and Sutton, D. S. 1997. Proceedings for the Pork Quality Summit. July 8 and 9. Des Moines, Iowa. p. 49–64.

Enfalt, A. C., Lundstrom, K., Lundkvist, L., Karisson, A., and Hansson, I. 1994. Technological meat quality and the frequency of the RN- gene in purebred Swedish Hampshire and Yorkshire pigs. 40th IcoMST.

Knipe, C. L., Olson, D. G., and Rust, R. E. 1988. Effects of inorganic phosphates and sodium hydroxide on the cooked cured color, pH and emulsion stability of reduced-sodium and conventional meat emulsions. *J. Food Science.* 53: 1305–1308.

Le Roy, P., Juin, H., Caritez, J. C., Billon, Y., Lagant, H., Elsen, J. M., and Sellier, P. 1996. Effet du genotype RN sur les qualites sensorielles de la viande de porc. *Jounees Rech. Porcine en France.* 28: 53–56.

Lundstrom, K., Andersson, A., and Hansson, I. 1996. Effect of the RN^- gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Science.* 42: 145–153.

Moiseev, I. V. and Cornforth, D. P. 1997. Sodium hydroxide and sodium tripolyphosphate effects on bind strength and sensory characteristics of restructured beef rolls. *Meat Science.* 45(1): 53–60.

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Effect of Different Garlic Products on Chinese-Style Sausage

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Abstract

Reduction of nitrite from 150 to 75 ppm resulted in an increase in total plate count organisms but no change in the oxidation of the product. No difference was found between fresh garlic, garlic powder, or essential oil of garlic for oxidation values or total plate count microorganisms, but oil of garlic resulted in the lowest acceptability scores. There was no evidence that addition of garlic could be used to reduce the bacterial level in sausage.

Introduction

Sausages are very common and popular processed meat products manufactured from lower-value trimmed meat to produce a higher-value product. Food additives are used to accomplish certain functions such as coloring, antimicrobial, antioxidative, preservation, improved nutrition, increased emulsification, and altered flavor. Garlic, which is available in three different forms (fresh, dehydrated, and extracted), is one of the most common spices which is frequently used in Chinese-style sausage. There are a lot of reports concerning the antibacterial and antioxidant effects of garlic on meat products (Juri-Haldeman *et al.*, 1987; Al-Delaimy and Barakat, 1970; Dewit *et al.*, 1978; Kourounakis and Rekka, 1991; Lin *et al.*, 1991; El-Khateib and El-Rahman, 1987; Lois, *et al.*, 1987; Ismaiel and Pierson, 1990). However, these effects have not been studied in Chinese-style sausage. Due to health concerns of some related to the reaction products of nitrite used in meat products and a desire to lower the level of nitrite, different forms of garlic were added to Chinese-style sausage to evaluate the flavor, color, antimicrobial, and antioxidant effects at two different nitrite levels (75 ppm and 150 ppm [normal]).

Materials and Methods

Boneless pork shoulders were trimmed (fat and lean trimmings separately) and ground through a 0.95 cm plate. Ground lean and fat were combined at a ratio of 4:1. Then, nitrite (75 or 150 ppm), salt (1.7%), seasoning (0.5% MSG, 8% sugar, 2% rice wine, 0.2% sodium polyphosphate) and spice (5% fresh garlic, 1.2% garlic powder, or 0.006% garlic essential oil) were added into the meat and thoroughly mixed. Farrell (1990) stated that "commercial oil of garlic, undiluted, has 200 times the strength of dehydrated garlic or 900 times the strength of fresh garlic." In this study, 5% of fresh garlic was chosen due to the results of the study of El-Khateib and El-Rahman (1987) who found that this quantity of garlic was more efficient at inhibiting bacteria than other treatments. And since 5% of fresh garlic has been reported to be equal to 0.006% undiluted commercial garlic oil and equal to 1.2% garlic powder, these levels were used as treatment levels in this research.

Seasoned meat was cured at 4ºC for one day for flavor and color formation and then stuffed into edible 2 cm diameter artificial collagen casings.

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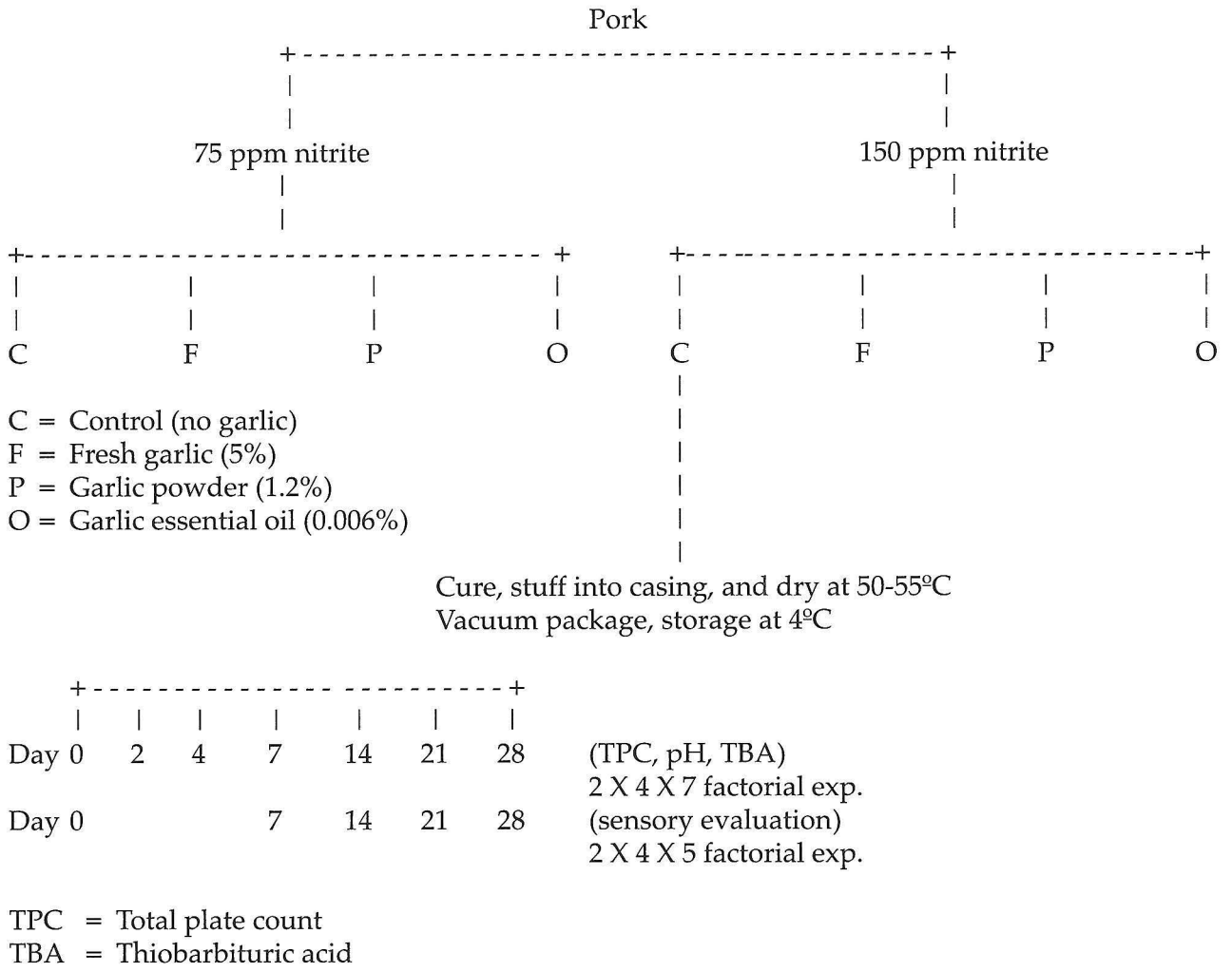

Figure 1. Experimental design.

Sausages were dried at 50 to 55ºC for 18 hours and then vacuum-packed in sterile bags and were stored at 4ºC.

The following evaluations were performed on the sausage products — thiobarbituric acid (TBA), total anaerobic plate count (TPC), pH, and sensory panel test (Figure 1). This is a 2 X 4 (X 5 or X 7) factorial experiment in a randomized complete block design. All the data were analyzed by analysis of variance (ANOVA) and significant difference determined by F-test (SAS, 1985). The means of treatments were evaluated by the Duncan procedures in the Statistical Analysis System (SAS, 1985).

Results and Discussion

The TBA Analysis

There was no significant two- or three-way interaction in TBA values, and no significance was found when comparing to 75-ppm and 150-ppm-nitrite-added groups (Table 1). These latter results were unexpected since nitrite has been reported as a very effective antioxidant in meat products (Ockerman, 1986); however, 75 ppm of nitrite may be sufficient for this purpose in vacuum-packaged products. There was also no significant difference due to garlic treatments, and again, this was unexpected since garlic has been reported to be a modest antioxidant in sausage items (Farrell, 1990). The TBA values also did not increase during the 28 days of storage, probably also due to the vacuum packaging. All the sausage samples indicated that there was little oxidation occurring with TBA values ranging from 0.15 to 0.20 g/kg. A value of 1.0 g/kg is usually considered to be the beginning of noticeable oxidation (Ockerman, 1986).

Table 1. Main Effects of Nitrite, Different Garlic Treatments, and Time on TBA (Thiobarbituric Acid) Values, TPC (Total Plate Count) Numbers, and pH Values on Chinese-Style Sausage.[1]

	Nitrite		Treatment			
	75 ppm	150 ppm	C	F	P	O
TBA	0.18[a]	0.18[a]	0.17a	0.20[a]	0.20[a]	0.17[a]
TPC[2]	4.36[a]	4.05[b]	4.24[a]	4.17[a]	4.29[a]	4.13[a]
pH	6.40[b]	6.43[a]	6.42[b]	6.45[a]	6.41[b]	6.39[c]

	Storage in Days						
	0	2	4	7	14	21	28
TBA	0.20[a]	0.20[a]	0.18[ab]	0.15[b]	0.18[ab]	0.15[b]	0.20[a]
TPC[2]	4.23[a]	4.33[a]	4.06[a]	4.26[a]	4.25[a]	4.12[a]	4.18[a]
pH	6.41[cd]	6.43[bc]	6.47[a]	6.45[ab]	6.39[d]	6.39[d]	6.38[d]

[1] C = control (no garlic), F = fresh garlic (5%), P = garlic powder (1.2%), O = Garlic essential oil (0.006%).
[2] TPC is expressed as CFU/g on a $\log_{10}$ basis.
[a,b,c] Means with different superscript letters in the same row for nitrite or treatment or storage time are significantly different ($P < 0.05$).

Total Plate Count (TPC) Numbers

No two- or three-way interaction was found for TPC numbers, and the 75-ppm-nitrite-added group had significantly higher TPC values than the 150-ppm-nitrite-added group as would be expected due to the antimicrobial effect of nitrite. There was no significant difference found for different garlic treatments, and no difference found during storage at 4ºC, both of which were unexpected since reports (Jurdi-Haldeman *et al.*, 1987) indicate that garlic has antimicrobial properties. However, the drying, nitrite, salt, wine, and vacuum packaging combination may help to inhibit bacteria growth. All the TPC numbers of the sausage samples were less than 10^6 CFU/g (an index number of spoilage [Wang, 1992]) and would suggest that microbial growth did not cause spoilage of these samples. Since TPC numbers decreased with nitrite level and were not different due to treatment, this would not suggest reducing the nitrite level in this product simply by adding garlic.

The pH Value

There was no two- or three-way interaction for pH values, and the group with 150-ppm nitrite had significantly higher pH values than that of the 75-ppm-nitrite-added group (Table 1). This was probably due to the greater growth of lactic acid bacteria in the 75-ppm nitrite treatment (Jay, 1991). The fresh garlic treatment resulted in higher pH values than all other treatments, with the garlic-essential-oil-added treatments having the lowest values. The pH values of all treatments showed no consistent change with storage time.

Sensory Evaluation

There was no two- or three-way interaction found among any sensory evaluation items, and

Table 2. Main Effects of Nitrite, Different Garlic Treatments, and Time on Sensory Properties of Chinese-Style Sausage.[1]

	Nitrite		Treatment			
	75 ppm	150 ppm	C	F	P	O
Red color[2]	5.49[a]	5.50[a]	5.50[a]	5.34[a]	5.44[a]	5.51[a]
Garlic flavor[3]	4.65[a]	4.76[a]	3.41[c]	6.77[a]	4.45[b]	4.19[b]
Juiciness[4]	5.72[a]	5.53[a]	5.54[a]	5.78[a]	5.60[a]	5.59[a]
Texture[5]	5.59[a]	5.46[a]	5.43[a]	5.60[a]	5.57[a]	5.49[a]
Oxidation[6]	6.28[a]	6.36[a]	6.21[a]	6.55[a]	6.29[a]	6.24[a]
Overall[7]	6.05[a]	5.92[a]	6.00[a]	6.08[a]	6.18[a]	5.69[b]

	Storage Time in Days				
	0	7	14	21	28
Red color[2]	5.97[a]	6.00[a]	5.25[b]	5.00[b]	5.24[b]
Garlic flavor[3]	5.26[a]	4.73[b]	4.52[bc]	4.10[c]	4.90[ab]
Juiciness[4]	5.83[a]	6.08[a]	5.84[a]	5.23[b]	5.14[b]
Texture[5]	5.78[b]	6.10[a]	5.71[b]	5.04[c]	4.98[c]
Oxidation[6]	6.83[ab]	6.95[a]	6.37[bc]	6.13[c]	5.32[d]
Overall[7]	6.26[ab]	6.56[a]	6.05[b]	5.65[c]	5.42[c]

1 C = control (no garlic), F = fresh garlic (5%), P = garlic powder (1.2%), O = Garlic essential oil (0.006%).
2 Red color: 1 = extremely dark, 9 =extremely light.
3 Garlic flavor: 1 = extremely bland, 9 = extremely garlic flavored.
4 Juiciness: 1 = extremely dry, 9 = extremely juicy.
5 Mouth feel texture: 1 = extremely fine, 9 = extremely coarse.
6 Oxidation: 1 = extremely rancid flavor, 9 = extremely nonrancid flavor.
7 Overall acceptability: 1 = very low overall acceptance, 9 = very high overall acceptance.
a,b,c,d Means with different superscript letters in the same row for nitrite, treatment, or time are significantly different ($P < 0.05$).

no differences were found between 150-ppm and 75-ppm-nitrite-added groups for any sensory evaluations (Table 2). This is not as would be expected, since nitrite has been reported to affect cured color and flavor, but in a dried, highly spiced product, 75 ppm of nitrite may be sufficient. No differences were found for color, juiciness, texture, or oxidation for the different garlic treatments. Fresh garlic treatment had a higher garlic flavor value than that of garlic powder and garlic essential oil treatments, and all the garlic treatments had higher garlic flavor than the control which would be expected. However, the garlic flavor for the garlic treatments should have been the same if the flavor ratio given in the literature was accurate (Farrell, 1990) or if the garlic used in this research was of average potency. The garlic-essential-oil treatment had lower overall acceptability values than the other three treatments. This was probably due to the oil extraction, where only a portion of the flavor profile of the fresh garlic was extracted, and this flavor might be unfamiliar or not as acceptable to some panelists. Also, garlic oil had a lower pH value than the other additives, which

might have given a bitter flavor. Garlic flavor strength maintained a fairly stable level during the storage period. However, the scores of all sensory items decreased slightly as storage time increased (Table 2) as would be expected.

Summary

No difference was found in yield, protein, and fat contents of both 75-ppm and 150-ppm nitrite groups. The utilization of 75 ppm of nitrite resulted in lower pH but higher TPC numbers than those of the 150 ppm nitrite-added group, but no significant difference in TBA values and sensory evaluation were found. The fresh garlic treatment showed higher pH and more intensive garlic flavor than the other treatments. No difference was found among all garlic treatments in TBA values and TPC numbers. No difference was found for all sensory evaluations due to garlic treatments except for the garlic-essential-oil treatment, which had a lower overall acceptability.

The pH value and sensory evaluation scores decreased with time, but no change was found in TBA values and TPC numbers during storage time.

Since bacteria numbers increased with lower nitrite levels and no improvement was found in TBA values or bacteria numbers with adding garlic, there is no evidence that adding garlic to Chinese-style sausage under conditions used in this research should be utilized as a reason to decrease the nitrite level.

References

Al-Delaimy, Kh. S. and Barakat, M. M. F. 1970. Antimicrobial and preservative activity of garlic on fresh ground camel meat — Effect of fresh ground garlic segments. *J. Sci. Fd. Agric.* 22:96-98.

Dewit, J. C., Notermans, S., Gorin, N., and Kampel Macher, E. H. 1978. Effect of garlic oil or onion oil on toxin production by *Clostridium botulinum* in meat slurry. *J. Food Prod.* 42(3): 222-224.

El-Khateib, T. and El-Rahman, H. A. 1987. Effect of garlic and *Lactobacillus plantarum* on growth of *Salmonella typhimurium* in Egyptian fresh sausage and beefburger. *J. Food Prot.* 50(4):310-311.

Farrell, K. T. 1990. *Spices, Condiments, and Seasonings.* 2nd Ed. Van Nostrand Reinhold, New York.

Ismaiel, A. and Pierson, M. D. 1990. Inhibition of growth and germination of *C. botulinum* 33A, 40B, and 1623E by essential oil of spices. *J. Food Sci.* 55(6):1676-1678.

Jay, J. M. 1991. *Modern Food Microbiology.* 4th Ed. Van Nostrand Reinhold, New York.

Jurdi-Haldeman, D., MacNeil, J. H., and Yared, D. M. 1987. Antioxidant activity of onion and garlic juices in stored cooked ground lamb. *J. Food Prot.* 50(5):411-413.

Kourounakis, P. N., Rekka, E. 1991. The activity of garlic powder and alliin as antioxidants and catchers of free radicals. *Medizinische Welt* 0(7A):44.

Lin, X., Li, I., Zhang, Q., and Mei, X. 1991. The preventive effect of garlic against toxicity of dimethyluitrosamine in rats fed with aminopyrine and nitrite. *ACTA Nutrimenta Sinica* 13(2): 126-132.

López Lois, A., Gutíerrez Fernández, L. M., Zumalacárregui Rodríguez, J. M., and Lopez Pérez, A. 1987. Changes in several constituents during the ripening — Chori20 — a Spanish dry sausage. *Meat Sci.* 19:169-177.

Ockerman, H. W. 1986. *Quality Control of Post-Mortem Muscle Tissue (1): Meat and Additives Analysis.* Department of Animal Sciences, The Ohio State University, Columbus.

SAS. 1985. *SAS User's Guide: Statistics.* 5th Ed. SAS Institute, Inc., Raleigh, N.C.

Wang, W. 1992. *Chinese traditional meat products and their processing.* 38th IcoMST v3, p 1154-1158.

Molds on *Jamón:* Selection of Strains and Optimization of Their Metabolic Activity

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Abstract

Dry-cured ham fungal flora is primarily composed of strains of the genus *Penicillium.* Twenty-two strains of this genus were evaluated under different conditions of temperature and relative humidity for their positive influence on flavor. The results indicated that two strains of *P. chrysogenum,* two strains of *P. camemberti,* and one strain of *P. aurantiogriseum* had a positive influence under all tested conditions. The best results from the technological point of view were obtained using *P. camemberti* and *P. aurantiogriseum* at low temperatures (3-8°C).

Introduction

Improving the technological basis for Spanish ham *(jamón)* production is a very important goal as this product is of high economic value to the meat-processing industry. One of the steps that influence the quality of *jamón* is the final aging of hams in a *bodega,* a traditional place, usually underground, very similar to wine cellars. During aging in the *bodega,* the *jamón* surface is usually covered by molds, a specific strain for each processing area.

Methods and Materials

A total of 40 hams from different geographical areas, which included most of the commercial *jamón* production in Spain, were analyzed and evaluated for types of mold, their influence on ham flavor, and the influence of temperature and humidity on the mold properties.

Results

This study concludes that the fungal flora is mainly composed of strains of the genus *Penicillium.* These results corroborate previous results of Rojas, 1994; Trigueros *et al.,* 1995; and Toledo *et al.,* 1997. Meat research workers Leistner and Ayres (1968), Leistner (1986), and Krotje (1992) reported that specific mold groups have a positive influence on the flavor development in meat products.

In this study, 22 isolated strains from the genus *Penicillium* that presented initial morphological differences were tested under different conditions of temperature and relative humidity for their positive influence on flavor. The initial results indicated that two strains of *P. chrysogenum,* two of *P. camemberti,* and one of *P. aurantiogriseum* have a positive influence on meat-product flavor under all of the tested combinations of temperature and relative humidity. These species have been isolated previously from meat products by Vayssier (1979), Huerta *et al.* (1987), and Rojas (1994).

This research also included a further metabolic study of the selected strains under different con-

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ditions of temperature and relative humidity. This was an attempt to obtain objective results in order to optimize the use of these microbial strains as starters in meat production.

The five selected strains have been studied for their influence on flavor in a model meat system (ground meat with 10% salt in the water phase added) simulating the surface of *jamón* during the *bodega* aging step. The study included changes in pH, NPN, free amino acids, ammonia N, acidity index of fat, peroxide index, and TBA index. The study also included monitoring the growth of bacterial populations (total microbial count and micrococci).

After analyzing the effects of conditions tested, it has been shown that time of incubation, inoculated strains, relative humidity, and temperature, as well as the interaction of most of these conditions, influenced the observed changes in the organoleptic and chemical studied parameters.

The best results from the technological point of view were obtained using *P. camemberti* and *P. aurantiogriseum* at low temperatures (3 to 8ºC).

References

Huerta, T., Sanchis, V., Hernández, J., and Hernández, E. (1987). *Mycoflora Mycrobiologie-Aliments-Nutrition.* 5: 247–252.

Krotje, D. 1992. International Food Ingredients 6:14–18.

Leistner, L. 1986. Mould ripened foods. *Fleischwirtsch* 66:1385–1388.

Leistner, L. and Ayres, J. C. 1968. Molds and meats. *Die Fleischwirtschaft* 48:62.

Rojas, F. J. 1994. *Evolución de la Flora Fúngica del Jamón Curado Durante su Elaboración: Influencia de los Conservadores.* Ph.D. Dissertation. Dpto Bromatología y Tecnología de Alimentos. Fac. Veterinaria. Univ. Córdoba, Spain.

Toledo, V. M., Selgas, M. D., Casas, M. C., Ordóñez, J. A., García, M. L. 1997. *Food Research and Technology* 204(5):385–390.

Trigueros, G., García, M. L., Ordóñez, J. A., y Selgas, M. D. 1995. Z. Lebensm. Unters. Forsch. 201:298–302.

Vayssier, Y. 1979. Microbial "fl. RTVA 18: 139.

Diffusion Coefficient of Salt During Meat Salting in the Production of *Jamón*

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Abstract

Dry salt penetration into muscle tissue is not a simple process with a constant diffusion coefficient. In contrast to expected results, the majority of the salt penetration occurs late in the salting process. This penetration rate could be due to the time for moisture to escape from the tissue to form the initial salt brine. Salt penetrates through capillary spaces, and at the same time salt can influence the tissue structure. It is important to monitor this process closely since a balance has to be maintained between minimum salt level for preservation and a maximum salt level for sensory purposes.

Introduction

Spanish traditional *jamón* is manufactured by salting and then drying and aging of pork hams for an adequate length of time to produce flavor. This process usually ranges from six months to two years. The most appreciated products are those prepared from hams of *Ibérico* pigs, which are aged for at least 18 months.

Processing of this type of ham *(jamón)* starts at the salting step. In this traditional process, the green hams are buried in dry salt and kept in a cold room for a variable length of time, based on the weight of the ham (i.e., x days per kg of green weight, with x being a specific value for each processor, depending on their historical empirical results). Using this procedure, it is common to see a great deal of variability among the same type of hams processed by the same processor. The hams with higher green weight usually contain more salt than the hams with the lower green weight (Crespo *et al.*, 1991). These results seem inappropriate since most of the final organoleptic characteristics in this product depend on the salt content and the additional desired requirement of getting the appropriate amount of salt into the final product.

The objective of this research is to establish a rational basis for the salting step of *jamón,* which includes the evaluation of the effective diffusion coefficient of salt during this processing step.

A recent reference attributes a unique diffusion coefficient for salt migration in ham processing that could be used to predict salt penetration during salting (Palmia, 1992). The model proposed has been tested against the results of experimental salt migration in *jamón.*

Material and Methods

A total of 27 hams were salted in dry salt according to the traditional Spanish method. First, the hams were rubbed with salt and then buried in three to four layers of dry salt. They were then maintained in a cool room at 3 to 5°C. Three hams were taken from the salting room every day during the first five days, and then at 7, 9, and 11 days.

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The salt penetration in hams was measured in cores of meat taken with a metallic hollow cylinder 3 cm in diameter, from the center of the lean meat part of the ham (under the coxal joint, close to the femur). These meat cylinders, with a mean length of 14 cm, were cut at regular depths of 2 cm, which resulted in seven equal sections. Each section was analyzed for moisture and salt content according to the procedure reported by Ockerman (1985).

The total amount of salt penetrating each cylinder at any time was evaluated as:

$$M_0 = \sum_{7}^{1} c_i * 2$$

with:

M_0 = total kilograms of salt that penetrate per square meter at the surface of the cylinder.

$c_{i.}$ = % (w/w) of salt in each "meat section" from 1 to 7.

The effective diffusion coefficient in each salting period was evaluated according to Crank (1957) from the total amount of salt penetrating per square meter at the evaluated zone:

$$M_0 = 2 * c_0 \sqrt{\frac{D*t}{\pi}}$$

with:

$$D = \frac{M_0^2 * \pi}{4 * c_0^2 * t}$$

and with:

c_0 = salt concentration at surface [263.4 kg/m^3- taken from (Ockerman, 1991)]

D = Diffusion coefficient (m^2/sec)

t = time (sec).

Results and Discussion

The results obtained in this study establish that salt penetration during the salting step is not a simple process with a constant diffusion coefficient.

The total amount of salt penetrating each day in *jamón* is presented in Figure 1. It is possible to see that that there is a complex penetration pattern and most of the salt penetrating the *jamón* tissue occurs at the end of the salting process. These are not the expected results since the differences in salt concentrations are much higher at the beginning of the salting process.

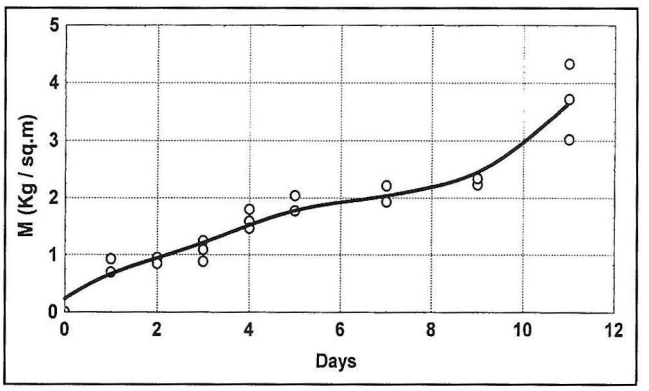

Figure 1. Total amount of salt penetrating in Spanish *Jamón* during salting.

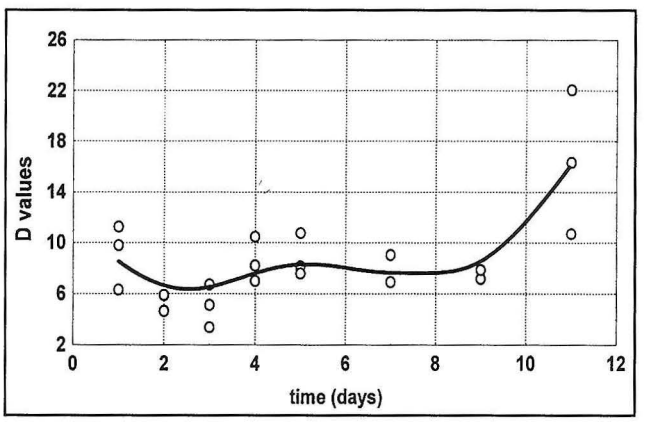

Figure 2. Apparent diffussion coeficient (D) of salt in the *Jamón* salting step.

To further clarify these results, the apparent D values were calculated and presented in Figure 2. It is possible to see that the D value changes during the salting step in a rather complex pattern.

The D values calculated from our results in the first day were lower than the normal effective diffusion coefficient found for fresh pork by Fox (1980) and were further reduced in the following days to very low values until the 11th day of salting. Then the effective coefficient of diffusion increased to values close to those found in the literature.

The low initial results might be due to the time required for the initial brine formation outside the ham tissue. Initial salt penetration in meat requires

that water from the product escapes from the ham to create a brine solution. According to Saravacos (1994), in salting food products, complex mechanisms operate. In addition to the simple diffusion due to the differences in concentration, salt diffuses into meat tissues due to osmotic processes in the membranes, flowing through capillary spaces between fibers and hydrodynamic flow (Bruin and Luyben, 1980). It is also necessary to realize that salt can influence the tissue structure (Raoult Wack, 1994).

In fact, the existence of "speeding paths" into meat tissues would explain the relative high D value for salt in meat vs. the D value for salt in water (Saravacos, 1994). As salt diffuses into the tissues, there is an interaction with meat proteins that increases their WHC (Hamm, 1960). As proteins hydrate, there is a "closing" of the paths for salt penetration, and a very low D value is obtained until the salt concentration reaches 6 to 8%. When the salt content further increases as diffusion proceeds, proteins shrink and the paths again open, increasing the D value.

These results are very significant in ham processing since it suggests that most of the salt penetration during ham salting occurs at the end of the salting process. Therefore, it is necessary to maintain close control at the end of the salting step, to be able to obtain at least the minimum salt level required for processing, as well as to avoid too much salt in *jamón*.

References

Bruin, S. and Luyben, K. Ch. 1980. Drying of food materials: A review of recent developments. In: *Advances in Drying*. A. S. Mujumdar, Ed. Hemisphere, N.Y.

Crank, J., 1957. *The Mathematics of Diffusion.* Oxford University Press.Hamm, R. 1960. *Advances in Food Research* 10:414.

Fox, J. B. 1980. *J. Food Sci.* 45:1740–1744.

León Crespo, F., Penedo Padron, J. C., Bandeira Velloso, C., Galan Soldevilla, H., Barranco Sanchez, A., Ciudad Gonzalez, N., and Peralta Fernandez, A. 1991. Distribution of salt in Spanish ham during the post-salting period. Proc. 37th Int. Cong. Meat Sci. Technol., Kulmbach; Vol III; 892–895.

Ockerman, H. W. 1985. *Quality Control of Post-Mortem Muscle Tissue.* 13th Ed. Department of Animal Sciences, The Ohio State University, Columbus, Ohio.

Ockerman, H. W. 1991. *Food Science Source Book.* VanNostrand Reinhold, New York.

Palmia, F., Mazoyer, C., Diaferia, C., Baldini, P., and Porretta, A., 1992. *Rev. Esp. Cienc. Tecnol. Aliment.* 32(1):71–83.

Raoult Wack, A. L. 1994. *Trends in Food Sci. Technol.* 5(8):255–260.

Saravacos, G. D. 1994. Mass transfer properties of foods. In: *Engineering Properties of Foods.* Rao and Rizvi, Ed. M. Decker.

Effects of Anka Rice, Nitrite, and Phosphate on Warmed-Over Flavor in Roast Beef

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Abstract

Warmed over flavor in cooked beef is primarily due to the oxidation of lipids, particularly phospholipids located in the lean tissue membranes. Anka rice did not inhibit lipid oxidation (thiobarbituric acid values) during surface curing but may have masked rancidity in cooked and cooled beef. But anka rice plus nitrite and phosphate (A+N+P) improved sensory evaluation and seemed to have a synergistic effect on flavor during refrigerated storage and proved to be the most desirable treatment for re-cooked beef.

Introduction

Warmed-over flavor (WOF) of cooked beef is primarily due to oxidation of lipids, particularly phospholipids located in lean tissue membranes. Many researchers have indicated that chelators help inhibit WOF in cooked beef. In the Orient, Chinese roasted pork is a very popular warmed-over food that uses deep dark-red anka rice as a coloring agent and to increase flavor.

The purposes of this study were to compare the effects of anka rice (A), phosphate (P), and nitrite (N) alone and in combination on WOF in roast beef; to compare the effects of these ingredients added in a curing solution only applied to the surface; and the effects on specific characteristics in roast beef during refrigerated storage.

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Materials and Methods

Experimental Design

The experimental design is shown in Figure 1. All treatment samples after cooking were stored at 4°C until measured for chemical analysis, oxidation, and physical characteristics at 0, 2, 4, 7, and 10 days of refrigerated storage after reheating, and microbial analysis was conducted on the same days without reheating. Sensory evaluation was tested at 0, 2, 4, and 7 days of refrigerated storage after reheating.

Chemical Analysis

Moisture analysis was performed by the oven drying method (Ockerman, 1985). Fat analysis was determined by the AOAC method of ether extraction (Ockerman, 1985). The pH values were measured on 1 to 10 diluted samples using a Corning pH meter (Ockerman, 1985).

Thiobarbituric Acid (TBA) Value

The samples of all treatments were separated into nonpeeled (whole tissue) and peeled (center tissue only) samples before the modified extraction/filtration TBA method (Pensel, 1990) was conducted.

Physical Measurements

Shear value was measured by the Warner-Bratzler shear instrument (Ockerman, 1985). The modified centrifuge technique (Tsai and Ocker-

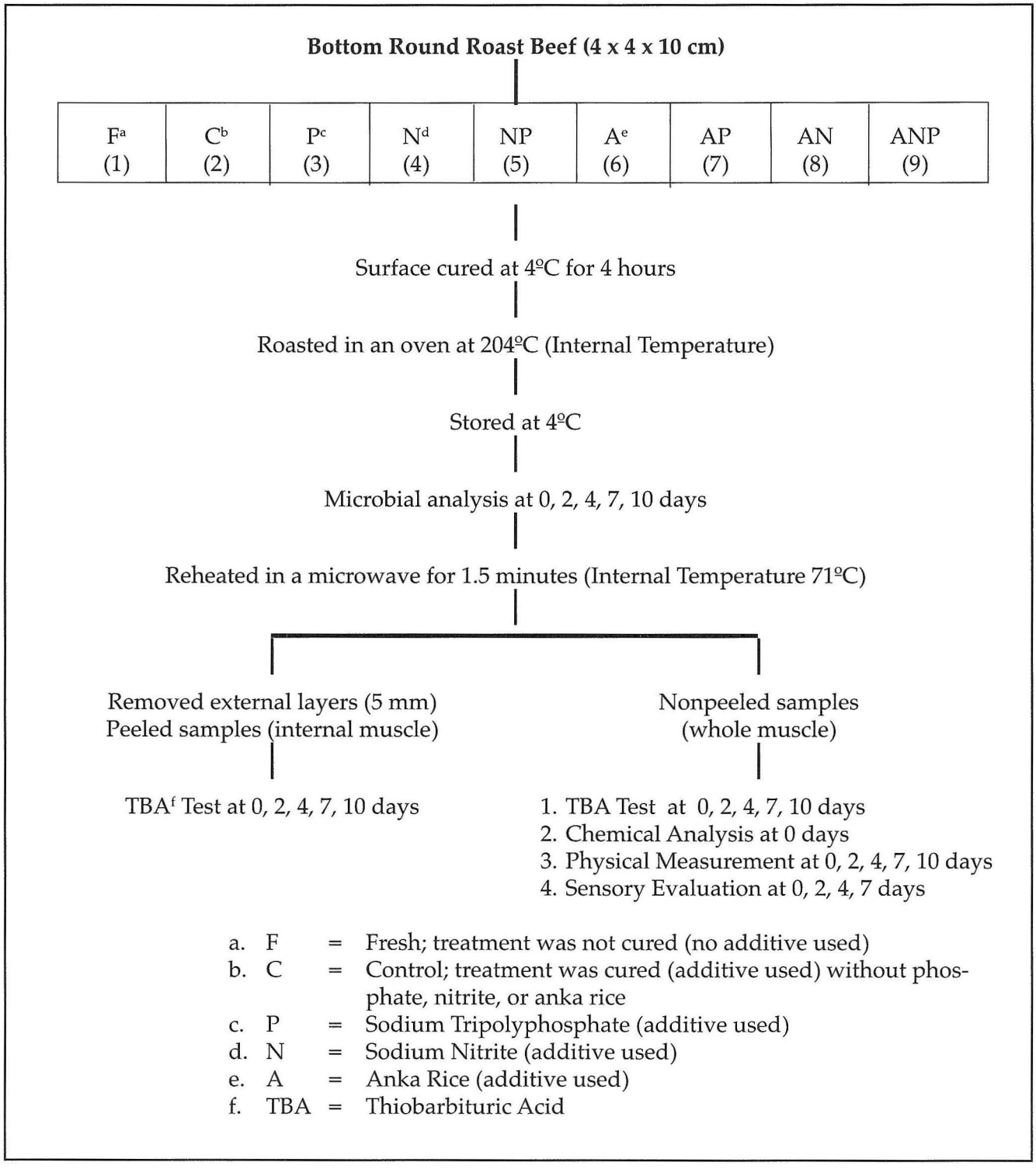

Figure 1. Experimental Design

man, 1981) was used to evaluate water-holding capacity (WHC).

Sensory Analysis

The sensory panelists were asked to score warmed-over flavor, warmed-over aroma, roast beef flavor, juiciness, and tenderness, utilizing a 9-point scale.

Total Plate Count (TPC) Method

TPC procedure (Speck, 1984) was used to measure microbial growth of samples, which were not reheated after refrigerated storage on the day of test.

Statistical Analysis

Data was analyzed by the Statistical Analysis System (SAS) to compute analysis of variance (ANOVA) and correlation coefficients (SAS, 1994). Duncan's multiple range test from SAS was used for comparison of treatment means and across times (SAS, 1994).

Results and Discussion

The Peeled and Nonpeeled TBA Values

The major tendency for peeled TBA values was a steady significant increase over time for all treatments (Table 1). The peeled TBA values of the fresh treatment were significantly higher than all others at four and seven days. At 10 days, the fresh and the control treatment had significantly higher TBA values than all other treatments. Of the other treatments, N and A+N treatments had the lowest peeled TBA values at 10 days. From two to 10 days, treatments that contained either P or N had significantly lower peeled TBA values when compared to the control treatment. When samples containing these two additives were compared at the levels used, it would also suggest that N added to treatments gives more antioxidative protection than P. However, the antioxidant activity of A treatment as measured by TBA values resulted in significantly higher TBA values when compared to both P and N treatments over these times (seven to 10 days) suggesting only minimal antioxidant

Table 1. Means of Peeled and Nonpeeled TBA[1] Values During Refrigerated Storage of Roast Beef.

	Peeled TBA					Nonpeeled TBA				
	Day					Day				
Treatments	0	2	4	7	10	0	2	4	7	10
Fresh	0.19^{bcE}	0.34^{abD}	0.52^{aC}	0.79^{aB}	0.97^{aA}	0.14^{eE}	0.39^{aD}	0.52^{aC}	0.84^{aB}	1.07^{aA}
Control	0.26^{aE}	0.37^{aD}	0.47^{bC}	0.64^{bB}	0.95^{aA}	0.22^{bcE}	0.37^{abD}	0.45^{bC}	0.75^{bB}	1.02^{bA}
Phosphate (P)	0.23^{aD}	0.26^{cdD}	0.37^{cdC}	0.55^{cdB}	0.76^{cA}	0.21^{bcdE}	0.26^{cD}	0.36^{cC}	0.55^{cB}	0.90^{dA}
Nitrite (N)	0.14^{dE}	0.27^{cD}	0.34^{dC}	0.45^{fB}	0.66^{dA}	0.20^{cdD}	0.26^{cC}	0.30^{eC}	0.41^{dB}	0.75^{fA}
N + P	0.14^{dE}	0.23^{dD}	0.34^{dC}	0.50^{eB}	0.75^{cA}	0.19^{cdE}	0.25^{cD}	0.32^{deC}	0.54^{cB}	0.86^{deA}
Anka rice (A)	0.23^{abE}	0.33^{bD}	0.41^{cC}	0.66^{bB}	0.85^{bA}	0.26^{aE}	0.36^{bD}	0.44^{bC}	0.76^{bB}	0.98^{cA}
A + P	0.25^{aD}	0.26^{cdD}	0.38^{cdC}	0.55^{cB}	0.75^{cA}	0.23^{abD}	0.24^{cD}	0.36^{cdC}	0.56^{cB}	0.85^{eA}
A + N	0.17^{cdE}	0.28^{cD}	0.34^{dC}	0.45^{fB}	0.66^{dA}	0.18^{dE}	0.26^{cD}	0.35^{cdC}	0.45^{dB}	0.76^{fA}
A + N + P	0.17^{cdE}	0.25^{cdD}	0.34^{dC}	0.52^{deB}	0.76^{cA}	0.23^{abD}	0.26^{cD}	0.34^{cdC}	0.55^{cB}	0.87^{deA}

[a,b,c,d,e,f] Means with different lowercase superscripts down the column are significantly different ($P < 0.05$).
[A,B,C,D,E] Means with different uppercase superscripts across the row in either peeled or nonpeeled categories are significantly different ($P < 0.05$).
[1] TBA value means as mg malonaldehyde/kg sample.

activity. N and A+N treatments maintained significantly lower peeled TBA values than other treatments — especially at seven and 10 days of storage.

For nonpeeled TBA values A+P and A+N+P treatments did not increase significantly during this time frame (two to 10 days). At zero day, the nonpeeled TBA value of the fresh treatment was lower than the control treatment, and the reason is probably due to the catalytic activity of some ingredients (e.g., salt) in the surface curing which increased lipid oxidation. In general, all treatments increased in TBA over storage times as would be expected. Again, fresh treatment received the highest value from two through 10 days of storage and this difference was significant from all treatments except the control treatment at two days. The next highest TBA values over time were for control and A treatments, and these were significantly higher than other treatments. However, at 10 days, the control treatment was even higher than the A treatment. Treatments with antioxidants (N and P but not A) had lower nonpeeled TBA values than the fresh and the control treatments from day two to 10. When N was added to treatments instead of P, the result would suggest that N had more antioxidative properties to decrease nonpeeled TBA values. From days seven and 10, N and A+N treatments received the lowest TBA values. At 10 days of refrigerated storage, the nonpeeled TBA value of anka rice treatment was closed to 1 mg/kg, and fresh and control treatments were higher than 1.0.

The correlation of peeled and nonpeeled TBA value was r = 0.97. The external surface has more extensive exposure to oxygen but also has more contact with additives, some of which should have antioxidative properties. In general, nonpeeled samples had higher TBA values, particularly late in storage, when compared to peeled samples, suggesting that oxygen content had a stronger influence on oxidation than antioxidative additives.

Treatments with N contained in surface curing had lower peeled and nonpeeled TBA values than those with P, and N treatment surprisingly was even lower in TBA values than the combination of these two ingredients. Antioxidants did not stop the increase of peeled and nonpeeled TBA values completely, but slowed down lipid oxidation. Overall, there was no synergistic effect of A, N, and P on TBA values in roast beef. In both peeled and nonpeeled TBA values, N and A were proven to be the most significant desirable treatments from a TBA standpoint.

Warmed-Over Aroma (WOA)

Lower WOA scores indicated more intense warmed-over aroma. At two days, the fresh, control, and A treatments had lower WOA scores compared to other treatments. The results suggested that A used alone did not inhibit WOA at two days, but N and P did retard the development of WOA. This agrees with the A treatment in nonpeeled samples, which also had similar TBA values as the control treatment, and other antioxidant treatments had lower TBA values compared to control treatment. From two to seven days, A used alone did not inhibit the development of WOA. This result also agreed with measurement of nonpeeled TBA values. At four days, WOA score of fresh treatment was lower than that of control treatment. The control treatment had the same score as other treatments except for N and A+N+P treatments. Treatments with antioxidants, except for the A treatment, had higher WOA scores than control treatment at seven days. Both P and N treatments decreased WOA at seven days. These results suggested that P and N inhibit the development of WOA in some cases, but that A did not.

WOA scores of all treatments decreased over refrigerated time; therefore, all antioxidative ingredients used in surface curing did not stop lipid oxidation but retarded its development. P, N, N+P, A+P, A+N, and A+N+P had more desirable WOA scores at seven days than did fresh, control, and A treatments. Only N and A+N+P treatments maintained higher WOA scores compared to control treatment from two to seven days. It would appear from both TBA values and warmed-over aroma results that N and P are retarding WOA development, and A may slightly mask it in some treatments.

Warmed-Over Flavor (WOF)

Lower WOF scores indicated more intense warmed-over flavor. Treatments with antioxidants (not including A treatment) had significantly more desirable WOF scores than the control treatment, which had values similar to fresh and A treatments at two days. A alone added in surface curing did

not inhibit the development of WOF during short storage (two days) when compared to the control. At the fourth day, only N and A+N+P treatments had significantly more desirable WOF scores when compared to the control, which had a higher score than the fresh treatment. Some ingredients used in the control treatment retarded or masked (flavoring ingredients) the development of WOF compared to fresh treatment at four days of refrigerated storage. At seven days of storage, all treatments containing nitrite and tripolyphosphate inhibited WOF development, but A did not. These results agreed with nonpeeled TBA values. All WOF scores decreased among treatments over time. Although N and P added in surface curing had more desirable WOF scores most days, they only retarded the development of WOF.

Other Characteristics

For Warner-Bratzler shear value, the fresh treatment had the significantly highest shear value over time. Some ingredients in the control treatment decreased shear value more than others. P at best had only a moderate effect on shear value due to surface curing. For water-holding capacity, A and N, in general, did not increase water-holding capacity in roast beef over refrigerated storage time. However, all the treatments with A, N, and P maintained one of the highest WHC values over time. There was a nonsignificant treatment-time interaction for roast-beef flavor, juiciness, and tenderness scores by sensory evaluation. All sensory scores of these three attributes significantly decreased during refrigerated storage of roast beef.

Microbial Growth (TPC)

N and A added in surface curing retarded microbial growth, but phosphate alone did not prevent it; however, it still inhibited microbes compared to the control treatment.

Conclusions

A did not inhibit lipid oxidation of roast beef as measured by TBA values but A+N+P often resulted in improved sensory evaluation (WOA and WOF) during refrigerated storage. This would suggest that under the conditions of this research A may have a flavor-masking effect on oxidation. Some characteristics of roast beef (WHC, shear value, juiciness, tenderness) also were not improved by A used in surface curing over time.

Sodium tripolyphosphate added in surface curing retarded lipid oxidation as compared to the control treatment. Due to surface-curing samples with added sodium tripolyphosphate, the pH, moisture, and yield were not significantly affected. However, objective parameters such as WHC and shear value or sensory evaluation such as juiciness and tenderness scores, were slightly improved by this alkaline phosphate in roast beef during refrigerated storage.

In several important characteristics, there seemed to be a synergistic effect among A, N, and P when used in surface curing and refrigerated storage. When comparing all evaluations, it currently would seem that the A+N+P is the most desirable treatment in retarding the development of warmed-over flavor and in retarding overall palatability of roast beef during refrigerated storage.

References

Ockerman, H. W. 1985. *Quality Control of Post-Mortem Muscle Tissue.* Department of Animal Sciences, The Ohio State University, Columbus, Ohio.

Pensel, N. A. 1990. *Influence of experimental conditions on porcine muscle and its effect on oxidation.* Thesis. The Ohio State University, Columbus, Ohio.

SAS. 1994. *Statistical Analysis System.* SAS Institute, Inc. Cary, N.C.

Speck, M. L. 1984. *Compendium of Methods for the Microbiological Examination of Foods.* 2nd Ed. American Health Association, Inc., Washington, D.C.

Tsai, T. C. and Ockerman, H. W. 1981. *J. Food. Sci.* 46:697.

Functional and Sensory Attributes of Normal pH Values in Sm Bull Muscles Depending on Time of Cutting and Aging

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Abstract

No influence was found for cutting time (24 and 48 hours post mortem [p.m.]) of the Semimembranosus (Sm) bulls' muscles and aging time (up 96 hours post mortem) on pH, cooking losses, dominant wavelength, color purity, firmness, and tenderness. Considering higher lightness after 48 hours p.m. and lower shear force after 48 and 72 hours p.m. for Semimembranosus muscles cut 24 hours p.m. than for those cut 72 hours p.m., it appears that muscles cut 24 hours p.m. should be directed to the retail trade. In further processing, it is necessary to take into account that the Semimembranosus muscle had the highest water-holding capacity after 48 hours, when cut 24 hours p.m., and after 72 and 96 hours p.m., when cut 48 hours p.m., which suggests a longer aging period for further processed product.

Introduction

The Longissimus dorsi muscle (Ld) is the most frequently used muscle for estimating meat quality attributes. The Ld and Semimembranosus (Sm) muscle differ with regard to muscle metabolism (O'Halloran *et al.*, 1997) and sensory properties (Carmack *et al.*, 1995; McKeith *et al.*, 1985). Only a few studies have focused on the physical and sensory properties of the Sm muscle of bulls. Some studies have included hot-boned and vacuum-packaging storage up to 15 days (Hertzman *et al.*, 1993; Olsson *et al.*, 1994), or cut from carcasses 7 days (Hostetler *et al.*, 1975) post mortem or cut 48 hours p.m. and then vacuum stored up to 14 days (Fjelkner-Modig and Ruderus, 1983).

It is common practice in meat processing to cut muscles from carcasses as early as possible. Polish legislation (8) requires that beef parts or cuts be aged no longer than 60 hours after excision at 24 hours p.m. and should be maintained at a temperature of 0 to 4°C and a relative humidity of 90%.

This research examined the influence of cutting time (24 and 48 hours p.m.) and aging time (up to 96 hours p.m.) on functional and sensory attributes of normal pH (pH < 5.8) Sm muscle of bulls.

Materials and Methods

Investigation was made on Sm muscles taken from young bulls 24 hours and 48 hours p.m. after commercial slaughter and rapidly cooled in a Wroclaw, Poland, meat factory. At different periods, the Sm muscles with a pH < 5.8 were excised from the hindquarter from six different bulls. Each time the Sm muscles were wrapped in polythene bags and transported in a cooler to the laboratory and then stored at 2 to 4°C for up to 96 hours p.m. After 24, 48, and 72 hours p.m., three 2.5 cm thick steaks were cut, and after 96 hours p.m., one steak was cut from the Sm muscles cut

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24 hours p.m. From Sm muscles cut 48 hours p.m., after 48, 72, and 96 hours p.m., three 2.5 cm thick steaks were cut. From the medium side of each steak, five to eight cores of 2 cm diameter and 1cm thick were obtained. The cores were then covered by oxygen-permeable polyvinyl-chloride film and allowed to bloom for 60 minutes at 2 to 4°C and utilized for lean-color analysis. The lean-color attributes:

Dominant wavelength:

$$lambda_{d\ (nm)} = 11.01 \times [(R_{640}/R_{540}) - 1] + 596.41$$

Color purity:

$$Pe_{(-)} = 0.105 \times \{0.1101\ [(R_{640}/R_{540}-1)^2 + 0.5641\ [(R_{640}/R_{540}) -1] + 1\} + 0.293$$

Lightness:

$$Y_{(\%)} = 1.029 \times R_{540} + 9.421$$

were estimated by measuring reflectance at 540 and 640 nm wavelengths with a Spectrophotometer (Specol) equipped with R 45/0 attachment according to the Tyszkiewicz (Tyszkiewicz, 1964) method. The remaining meat was ground through a 3 mm plate. The pH was measured with a meter by electrodes inserted into the ground meat. Water-holding capacity (WHC) was expressed as percent of bound water (Wierbicki *et al.*, 1962). A meat homogenate was obtained by homogenization of 15 g of ground meat with 60 ml of 0.67 M NaCl at pH 6.5 for 1 min at 4,000 rpm. It was used for determination of viscosity (Pa x s) at a shear rate of Dr 16.2 (s-1) with a Rotatory viscometer "Rheotest-2" with attachment H.

The two remaining steaks in separate plastic bags were cooked in a water-bath at 80°C for 90 minutes, cooled for 40 minutes, and weight loss was reported as percent cooking loss. After refrigeration for 18 hours, the steaks were cut into six sections (2 cm^2) for sensory evaluation and into six to eight slices (3 x 2 x 2 cm length x width x depth) for Warner-Bratzler peak shear force (kg). Samples were evaluated by six trained judges based on a 7-point scale for flavor, 1 = extremely strong, 7 = extremely weak; juiciness, 1 = extremely juicy, 7 = extremely dry; firmness, 1 = extremely weak (soft), 7 = extremely firm; and tenderness, 1 = extremely tender, 7 = extremely tough.

For each of six animals studied, multiple determinations were obtained and analysis of variance (ANOVA) and Duncan's method were used to test differences (11).

Results

Sm muscles cut 24 and 48 hours p.m. which had an ultimate pH lower than 5.8 were treated as normal meat. The pH of Sm muscles cut both at 24 and 48 hours p.m. did not change significantly during the aging time (Table 1). A lack of pH change during aging was not reflected in WHC. Muscles cut 24 hours p.m. and after 48 and 96 hours p.m. storage had the highest WHC and were significantly different from minimal WHC after 72 hours p.m. For the Sm muscle cut 48 hours p.m., WHC significantly increased after 72 hours p.m., and a further increase of WHC was not significantly different from the value of the WHC after 72 hours p.m. WHC of Sm muscles cut 48 hours p.m. in relation to the WHC of Sm muscles cut 24 hours p.m. and after 72 hours was significantly higher by 4.57%. No significant difference in cooking losses from Sm muscles cut 24 and 48 hours p.m. during aging were found despite significant changes in WHC of these muscles (Table 1).

The lowest meat homogenate viscosity for Sm muscle cut after 24 and 48 hours p.m. was found after 72 hours p.m. of storage (Table 1). Viscosity of Sm muscles cut 24 hours p.m. compared to the viscosity of Sm muscles cut 48 hours p.m. was significantly higher (2.84 to 4.31 times) at the corresponding storage times. Based on the Borderias *et al.* (Borderias *et al.*, 1985) hypothesis, the changes in viscosity can be explained by different actomyosin solubility. The lower meat homogenate viscosity for Sm muscles cut 48 hours p.m. than for Sm muscles cut 24 hours p.m. can be explained by lower amounts of extractable actomyosin from these muscles.

The color parameters (dominant wavelength and color purity) for Sm muscles cut 24 and 48 hours p.m. did not change significantly during aging and were comparable for corresponding storage times (Table 1). However, lightness of Sm muscles cut 48 hours p.m. increased during aging and after 96 hours p.m. the value was significantly higher than after cutting 48 hours p.m. Moreover, lightness of the Sm muscles cut 24 hours p.m. and

after 48 hours p.m. was higher by 1.42% than for the Sm muscles after cutting 24 hours p.m. and after 48 hours p.m.

Within each muscle group up to 72 hours p.m. when cut 24 hours p.m., and up to 96 hours p.m. when cut 48 hours p.m., there were no significant differences in sensory attributes with only one exception (Table 1). The flavor intensity of steaks from Sm muscles cut 48 hours p.m. decreased during storage and after 96 hours p.m. was significantly different than after cutting 48 hours p.m. Moreover, the steaks from Sm muscles after cut-

Table 1. Means and Standard Deviations (sd) for Functional Properties and Color Parameters of Semimembranosus (Sm) Muscles Cut 24 Hours Post Mortem (p.m.) and 48 Hours p.m. from Bull Carcasses and After Thermal Treatment for Shear Force and Sensory Characteristics of Steaks During Cold Aging Up to 96 Hours p.m.[1]

Parameter	Aging Time (Hours Post Mortem)						
	Sm Muscles Cut 24 Hours p.m.				Sm Muscles Cut 48 Hours p.m.		
	24	48	72	96	48	72	96
pH	5.40 (0.4)	5.45 (0.05)	5.48 (0.01)	5.47 (0.01)	5.45 (0.04)	5.50 (0.05)	5.51 (0.04)
WHC[2], %	5.96^{abc} (1.72)	8.25^{bcd} (0.97)	4.25^{a} (1.36)	7.96^{bcd} (0.81)	5.30^{ab} (1.89)	8.82^{cd} (3.69)	9.49^{d} (1.65)
Viscosity of meat homog., Pa x s	255.88^{ab} (39.93)	270.27^{a} (51.59)	223.94^{b} (36.53)	231.08^{ab} (18.06)	95.12^{c} (16.20)	51.60^{d} (17.27)	62.30^{c} (17.52)
Cooking losses, %	42.07 (0.99)	42.32 (1.29)	42.50 (1.27)	— —	41.95 (0.94)	41.11 (1.28)	41.33 (0.61)
Dominant wavelength (lambda d), nm	635.71 (3.17)	635.52 (3.66)	639.19 (2.54)	636.53 (2.42)	637.88 (3.72)	639.79 (2.46)	637.00 (2.92)
Color Purity (Pe), -	0.761 (0.057)	0.756 (0.047)	0.804 (0.034)	0.768 (0.031)	0.784 (0.050)	0.812 (0.034)	0.777 (0.039)
Lightness (Y), %	16.14^{ab} (1.30)	17.04^{a} (1.45)	16.34^{ab} (0.85)	16.95^{a} (0.54)	15.62^{b} (0.93)	16.16^{ab} (1.24)	17.50^{a} (0.65)
Flavor	3.85^{ab} (0.63)	3.75^{abc} (0.68)	4.35a (0.41)	— —	3.14^{c} (0.69	3.55^{bc} (0.73)	4.17^{ab} (0.75)
Juiciness	4.35^{ac} (0.82)	3.90^{c} (0.84)	4.55^{abc} (0.69)	— —	5.36^{b} (0.85)	4.80^{ab} (0.92)	4.75^{abc} (0.76)
Firmness	5.72 (0.57)	5.40 (0.52)	5.36 (0.67)	— —	5.42 (0.67	5.38 (0.52)	5.00 (0.00)
Tenderness	4.83 (1.25)	4.46 (1.08)	4.05 (0.44)	— —	5.14 (0.6	5.21 (0.62)	4.75 (0.99
Shear Force (SF)	9.09^{ab} (2.45)	8.42^{b} (2.25)	7.49^{b} (1.47)	— —	10.98^{a} (2.27)	10.51^{a} (2.02)	10.78^{a} (1.50)

[1] Means with different superscripts in the same row are significantly different ($P < 0.05$).
[2] WHC = Water-holding capacity.

ting 48 hours p.m. were estimated as having significantly higher flavor intensity than samples from the Sm muscles after cutting 24 hours p.m. and after aging for 72 hours p.m. The same relationship was found when comparing flavor after 72 hours p.m. of storage for the Sm muscle cut 24 and 48 hours p.m. respectively.

On the other hand, steaks from the Sm muscles after cutting 48 hours p.m. were estimated as being less juicy than steaks from the Sm muscles after cutting 24 hours p.m. and after aging 48 hours p.m. These differences in juiciness evaluation of steaks did not correspond with the WHC values, which, for corresponding periods, were not significantly different.

The shear force (SF), which was lower within all examined periods for steaks from the Sm muscles cut 24 hours p.m. than for steaks from the Sm muscles cut 48 hours p.m., was not reflected by sensory estimation of the Sm steaks' tenderness, which was not significantly different for both cutting and aging times.

Discussion

The lower lightness of Sm muscles after cutting 48 hours p.m. than after aging up to 96 hours p.m. (Table 1) is consistent with results of Griffin *et al.* (Griffin *et al.*, 1982) who found that the Sm of beef muscles after cutting three days p.m. had less brighter lean color than after nine days of muscle slices vacuum stored.

The flavor intensity decrease found in steaks from the Sm muscles cut 48 hours p.m. (Table 1) during storage is not in agreement with findings of Fjelkner-Modig and Ruderus (1983) who found that steaks from the Sm bull muscles cut 48 hours p.m. and in slices vacuum stored were estimated as having more intense flavor after five and 14 days. Although some authors found that flavor of steaks from the Sm beef muscles, hot boned, sliced, and placed in vacuum storage, was optimal after four days and after that was less intense (Spanier *et al.*, 1997), the Sm steer muscles were more desirable when muscles were cut from aged carcasses after four rather than after seven days p.m. (Parrish *et al.*, 1969), but these results were not obtained from bulls.

Some authors have shown a lack of significant differences in juiciness (Carmack *et al.*, 1995) and SF of (Hostetler *et al.*, 1975; Fjelkner-Modig and Ruderus, 1983; Parrish *et al.*, 1969) steaks from bulls' or steers' muscles after cutting and/or additional aging which agrees with results for juiciness and SF presented in Table 1. However, the others found more desirable juiciness of steaks from the Sm steer muscles after four rather than after seven days of carcass aging (Parrish *et al.*, 1969) and decrease of SF in steaks from the Sm bull muscles hot boned during vacuum storage up to 15 days (Hertzman *et al.*, 1993; Olsson *et al.*, 1994) or from Sm beef muscles after 11 days of aging wholesale cuts (Smith *et al.*, 1978).

Insignificant changes in tenderness of steaks from the Sm muscles cut both 24 and 48 hours p.m. during aging (Table 1) are not consistent with results of Fjelkner-Modig and Ruderus (1983) who found an improvement in tenderness of steaks from the Sm bull muscles cut 48 hours p.m. during vacuum aging and results of Parrish *et al.* (1969) who found that tenderness of steaks from the Sm steer muscles were more desirable after four rather than after seven days of carcass aging.

Conclusions

No influence was found for cutting time (24 and 48 hours p.m.) of the Sm bull muscles and aging time (up 96 hours p.m.) on pH, cooking losses, dominant wavelength, color purity, firmness, and tenderness. Considering higher lightness after 48 hours p.m. and lower SF after 48 and 72 hours p.m. for Sm muscles cut 24 hours p.m. than for those cut 72 hours p.m., it appears that muscles cut 24 hours p.m. should be directed to the retail trade. In further processing, it is necessary to take into account that the Sm muscle had the highest WHC after 48 hours, when cut 24 hours p.m., and after 72 and 96 hours p.m., when cut 48 hours p.m.

References

Javier Borderias, A., Jimenez-Colmenero, F., and Tejada, M. 1985. *J. Food Technol.* 20:31-42.

Carmack, C. F., Kastner, C. L., Dikeman, M. E., Schwenke, J. R., and Garcia Zepeda, C. M. 1995. *Meat Science* 39:143–147.

Fjelkner-Modig, S. and Ruderus, H. 1983. *Meat Science* 8:203–220.

Griffin, D. B., Savell, J. W., Smith, G. C., Lind, K. D., and Galloway, D. E. 1982. *J. Food Sci.* 47:1746–1748.

Hertzman, C., Olsson, U., and Tornberg, E. 1993. *Meat Science* 35:119–141.

Hostetler, R. L., Carpenter, Z. L., Smith, G. C., and Dutson, T. R. 1975. *J. Food Sci.* 40: 223–226.

Mc Keith, F. K., De Vol, D. L., Miles, R. S., Bechtel, P. J., and Carr, T. R. 1985. *J. Food Sci.* 50:869–872

O'Halloran, G. R., Troy, D. J. and Buckley, D. J. 1997. *Meat Science* 45:239–251.

Oktaba, Elementy statystyki matematycznej I metodyka doswiadczalnictwa. 1980. Ed. by Warszawa, P. W. E .

Olsson, U., Hertzman, C., and E. Tornberg, 1994. *Meat Science* 37:115–131.

Parrish Jr., F. C., Rust, R. E., Popenhagen, G. R., and Miner, B. E. 1969. *J. Animal Sci.* 29:398–403.

Polska Norma. 1988. PN-88/A–82003.

Smith, G. C., Culp, G. R., and Carpenter, Z. L. 1978. *J. Food Sci.* 43:823–826.

Spanier, A. M., Flores, M., McMillin, K. W., and T. D. Bidner. 1997. *Food Chemistry* 59:531–538.

Tyszkiewicz, St. 1964. *Roczniki IPMiT* 1:51–77.

Wierbicki, E., Tiede, M.G., and Burrell, R. C. 1962. *Fleischwirtschaft* 10:948–951.

Specific Characteristics of Meat from the *Ibérico* Breed

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Abstract

Strain of pigs, feeding program, animal age, and production environment had a significant influence on many biochemical properties on dry cured hams studied in this research.

Introduction

Meat from the *Ibérico* breed of hogs presents different characteristics from commercial pork. The *Ibérico* pigs are aged animals, always castrated, and grown under extensive production practices. The breed effect is considered to be very important by Spanish pig producers, who maintain their own breeding programs and register books (Dieguez Carbayo, 1992). Within the *Ibérico* breed, it is possible to distinguish many strains that present differences in results of commercial cut yields (Aparicio Macarro, 1987).

The *Ibérico* pigs are usually grown to a minimum of 12 to 18 months compared to commercial pork that is slaughtered at five to seven months. Therefore, a specific effect due to age is present. As the animal gets older, there is an increase in oxidative metabolism in the muscle fibers, increasing the color of meat and the tendency toward "dark, firm, and dry" (DFD) muscle tissue (Lawrie, 1974). The traditional production of this breed imposes castration which slows growth, reduces efficiency, and makes the carcasses fatter (Gerrard and Mallion, 1977). Due to the normal exercise of these animals that are feed in fields, there is a higher vascular system development in their muscles, resulting in increased blood supply.

Materials and Methods

This study included a total of 699 animals from five different strains of the *Ibérico* breed, which were grouped into lots in *montanera* (mountain) areas. They were fed commercial feed (feed lot) or combined natural feed plus feed lot, slaughtered at different times (from 12 to 18 months of age), and grown in different environments (four different farms, during a three-year span).

Results and Discussion

Meat samples taken from the *Psoas* muscle were analyzed for moisture, protein, fat, ash, phosphate, pH, NPN, total water-soluble proteins, total soluble pigments, CRA index (compression on filter paper method), and thiobarbituric acid (TBA) index.

Strain had a significant effect on most of the studied parameters except for total fat, total phosphate, CRA index, and TBA index. Feeding type influenced all the studied parameters except total pigments. Age and production environment had a significant effect on all the studied parameters.

The mean values of the studied parameters are shown in Table 1.

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References

Aparicio Macarro, J. 1987. *El Cerdo Ibérico.* Sanchez Romero Carvajal Jabugo S.A.

Dieguez Carbayo, E. 1992. Historia, evolución y situación actual del cerdo ibérico. In: *El Cerdo Ibérico, la Naturaleza, la Dehesa.* Ed. MAPA, Madrid.

Gerrard, F. and Mallion, F. J., Ed. 1977. *The Complete Book of Meat.* Virtue, Surrey, U.K.

Lawrie, R. A. 1974. *Meat Science.* 2nd Ed. Pergamon Press, New York.

Table 1. Mean Values of Meat from the *Ibérico* Pigs.[1]

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Phosphate (mg/g)	pH	NPN (%)	Water Soluble Proteins (%)	Total Pigments (OD)	CRA Index (1/S)	TBA Index (OD)
Number of Animals	699	699	699	699	699	699	699	699	699	699	699
Mean	72.29	26.16	0.96	1.02	238	6.06	0.27	7.47	0.188	0.737	0.770
SD	1.87	1.40	0.87	0.15	35	0.29	0.07	1.25	0.041	0.205	0.440

[1] NPN = Non-protein nitrogen, OD = optical density, CRA index = compression on filter paper method, TBA = thiobarbituric acid, and SD = standard deviation.

The Evaluation of the Palatability of a Dehydrated Meat Product-Meat Floss

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Abstract

A modified more rapid and less expensive process was developed to produce a palatable and tender dehydrated pork item. This cooked product will keep without refrigeration and will not drastically change with room-temperature storage. Due to the low moisture content, there were few microorganisms and mold growth, and this aided in extending the shelf life. A low-fat version was produced along with a more flavorful, higher fat version. This product could be used as a snack item or incorporated into other food items to add flavor and increase the protein content.

Introduction

Meat is well known as an excellent protein and energy source for our daily diets and, after digestion, provides excellent nutrition. However, meat can be very perishable due to its high moisture and protein contents, which may be utilized by microorganisms. The principle of extending the shelf life of meat products is to produce an unfavorable environment for microorganisms to grow. Among many preservative methods, dehydration was probably one of the earliest and most effective methods that has been developed.

In this project, a new type of oriental-style dehydrated meat product-meat floss, also called shredded pork (Chang and Huang, 1991), which possesses the advantages of long shelf life, desirable taste, safe ingredients, and low cost was evaluated. Of all the oriental dehydrated meat products, shredded pork is probably the most important fresh meat substitute in areas of China where refrigeration is not available. Its amazing long shelf life at room-temperature storage and good nutritional values have also brought great convenience to travelers and campers. It may also serve as a protein sustenance for the military, because it also has the advantages of being lightweight, easy-to-pack, and ready-to-eat. It can also serve as a snack or combine with other foods as part of the daily diet for the general population.

In this project, a less expensive meat source, the shoulder instead of traditionally used ham, and a new less labor-extensive processing technique with variable fat levels (2 and 12%) were compared with the time and labor-intensive traditional processing method. Since little is known about this oriental product, chemical analysis (fat, protein, and moisture), biochemical properties (pH and thiobarbituric acid [TBA]), microbiological assays (total plate count and mold growth), and sensory evaluation (color, meaty flavor, rancidity, texture, and overall acceptance) were conducted to investigate the potentials of this dehydrated meat product.

Experimental Design

The experiment was designed as a 2 (fresh and frozen) x 2 (traditional and modified) x 2 (2% and 12% lard) factorial. Boneless, skinless pork shoul-

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ders were obtained from a local supermarket in Columbus, Ohio. Eight approximately 5.4 pounds (2.45 kg) of boneless, skinless pork shoulders were prepared for trimming. All visible fat and connective tissue were carefully removed from the surface and internal tissue. The trimmed lean pork was then prepared for either the traditional or the modified process. The procedure for traditional method included moist-cooking (100°C, 3-1/3 hours), physical fiber separation, moisture evaporation (100°C, 55 minutes), and stir-frying (60 to 65 strokes/minute for 55 minutes). The modified procedure was autoclave (121°C, 15 psi, 30 minutes), physical fiber separation, moisture evaporation (100°C, 55 minutes), stir-frying (60 to 65 strokes/minute for 29 minutes) combined with 15 minutes of hot-air drying, and then convection-oven dried (93.3°C, 45 minutes). All samples were vacuum-packaged and stored in a dark room at room temperature (27 ± 2°C).

Cooking yield, moisture, crude fat, and crude protein contents were established at week 0. Since the pH, TBA, and sensory evaluation (color, meaty flavor, rancidity, texture, and overall acceptance), total plate count, and mold count were measured at week 0, 1, 3, 5, and 7 of storage, the storage time also became an additional independent variable (the fourth main effect).

Result and Discussion

The results of all measurements are summarized in Table 1. For cooking yield and chemical analysis, there was no significant three- or two-way interaction between the main effects ($P < 0.05$). The results of cooking yield (%) are shown in Table 1. The 2% lard addition treatments (44.81%) are significantly lower than those with 12% lard addition treatments (54.68%) at the 0.05 level. This suggests that the level of lard addition plays an essential role in the cooking yield.

For moisture content analysis, the treatments with 12% of lard addition had a lower percentage of moisture content (3.47%) than those with 2% of lard addition (5.23%). This was caused by the increased level of lard (fat), which will alter the proportions of other components (moisture, protein, and fat) in the meat products. In addition, it indicates that temperature of raw material and cooking method have no significant influence on the moisture content of the meat floss. The same trends are shown in crude fat and crude protein (Table 1). The pH values show little or no changes during seven weeks of room temperature (27 ± 2°C) storage. It supports the hypothesis that meat floss is a very shelf-stable product.

On the other hand, the data indicates that there are a few statistical differences between some of the three- or two-way treatments. However, these influences are very minor. The TBA value has been commonly considered as an index of lipid rancidity. Table 1 indicates that the TBA values slightly increased during storage (0.45 to 0.63 μg/g). The oxygen in the vacuum-packed bags could have come from the slow rate of natural release from the internal air cells of the meat floss. As Ockerman (1985) indicated, a TBA number of 1.0 is considered as the threshold level for rancidity in pork by some processors. The results also suggest that a longer than seven weeks of product shelf life at room temperature (27 ± 2°C) storage may be expected. Color scores for all treatments were very consistent during the seven weeks of storage.

Results indicate that the level of lard addition is the main effect that influences the meaty flavor (Table 1). The results were as expected, since fat is the main factor responsible for the volatile flavor compounds of meat products. Due to more macromolecules in meat products being degraded into smaller ones during storage, the trend of a slight increase in meaty flavor was noted. The slight increase of the rancidity score during the seven weeks of storage may be caused by the same reason described for the TBA values. There was no significant difference at the 0.05 level in the texture scores during storage. The panel's response to texture was "somewhat crispy."

Overall acceptance indicates that three main factors, raw material temperature, lard addition, and storage time had no significant effect on the evaluation. However, a fourth main effect, the cooking method, showed that panels preferred the modified method (6.00) over the traditional method (5.88), despite the fact there was only a slight difference between these two techniques. For microbial assays, the $\log_{10}$ TPC numbers were very low (2.01 to 2.18), which indicated that the numbers of microbial flora were lower than 200/g, even after seven weeks. The results of $\log_{10}$ colony forming units (CFU) mold count were very similar to

Table 1. Statistical Significance ($P < 0.05$) of Main Effects for Cooking Yield, Chemical Analysis, pH, Thiobarbituric Acid (TBA) Values, Sensory Evaluation, and Microbial Assays.

Treatment (Main Effects)	Raw Material Temperature		Cooking Method		Lard Addition		Storage Time				
	Fresh (4 ± 2°C)	Frozen (-18 ± 2°C)	Traditional	Modified	2%	12%	Week 0	Week 1	Week 3	Week 5	Week 7
Cooking Yield[1],%	49.94[a]	49.54[a]	49.39[a]	50.09[a]	44.81[a]	54.68[b]	NA[5]				
Moisture[1], %	4.37[a]	4.33[a]	4.35[a]	4.35[a]	5.23[a]	3.47[b]	NA				
Crude Fat[1], %	24.15[a]	24.07[a]	24.15[a]	24.06[a]	16.89[a]	31.33[b]	NA				
Crude Protein[1], %	38.71[a]	38.28[a]	38.50[a]	38.49[a]	42.90[a]	34.09[b]	NA				
pH[2]	—	—	—	—	—	—	6.33[a]	6.33[a]	6.36[a]	6.34[a]	6.33[a]
TBA[2],μg/g	—	—	—	—	—	—	0.45[a]	0.53[b]	0.54[bc]	0.56[c]	0.63[d]
Color[3]	—	—	—	—	—	—	4.55[a]	4.39[a]	4.29[a]	4.48[a]	4.44[a]
Meaty Flavor[3]	5.51[a]	5.50[a]	5.46[a]	5.55[a]	5.32[a]	5.70[b]	5.48[a]	5.52[a]	5.37[a]	5.51[a]	5.86[b]
Rancidity[3]	3.04[a]	3.18[a]	3.11[a]	3.10[a]	3.05[a]	3.16[a]	2.92[a]	3.01[a]	3.04[ab]	3.23[ab]	3.34[b]
Texture[3]	—	—	—	—	—	—	5.70[a]	5.73[a]	5.60[a]	5.72[a]	5.64[a]
Overall Acceptance[3]	5.86[a]	6.02[a]	5.82[a]	6.07[b]	5.88[a]	6.00[a]	5.88[a]	6.02[a]	5.93[a]	5.87[a]	6.00[a]
TPC[4]	—	—	—	—	—	—	2.18[a]	2.04[a]	2.08[a]	2.12[a]	2.01[a]
Mold Count[4]	—	—	—	—	—	—	0.00[a]	1.47[b]	1.27[b]	0.81[c]	0.64[c]

a,b,c, and d: Means with different superscripts in the same attribute row indicate the significant difference at the 0.05 level ($P < 0.05$) — indicates three- or two-way interactions were involved; therefore, they are not applicable in this table. N/A indicates the main factor (storage time) is not applicable in three-way (Temperature x Cooking Method x Lard Addition) model.

[1] Indicates the cooking yield and chemical analysis were done at week 0. n = 48.

[2] Indicates pH and TBA were done at week 0, 1, 3, 5 and 7. n = 240.

[3] Indicates sensory evaluations were done at week 0, 1, 3, 5, and 7. Trained panel number = 10. n = 800. The 9-point scale of sensory panel scores for each attribute are: 1 = *very light, bland or no, fluffy,* and *dislike extremely;* 5 = *medium or moderate or neither like nor dislike;* 9 = *very dark, intense or pronounced, crispy, or like extremely.*

[4] Indicates the microbial assays were done at week 0, 1, 3, 5, and 7. Total plate count (TPC) and mold count were expressed as $\log_{10}$ CFU/g. n = 960.

[5] NA = Not available.

the results in TPC; both indicated extremely low microbial growth. It indicates that the Log_{10} CFU/g for storage-time treatments were very low (0.00 to 1.47), which means that the numbers of mold colonies were lower than 30/g during the entire seven weeks of storage. It also indicates the unfavorable growing environment (low moisture) is not suitable for bacteria or mold growth.

Conclusion

- Lard-addition level is the major factor that influenced the cooking yield, chemical analysis (moisture, crude fat, and crude protein), and meaty flavor. Raw material and processing method had no significant influence on the cooking yield and chemical analysis.
- The pH, color, texture, and overall acceptance did not change during storage.
- The TBA value and rancidity score were slightly increased by storage time but were not objectionable. Rancidity was not significantly affected by raw-material temperature or processing technique. The relationship between TBA and rancidity scores is:

$$Y_i = 0.3518\ X_i - 0.5508,$$

where X = rancidity scores, Y = TBA value, i = Week 0, 1, 3, 5, and 7 (R^2=0.87).

- The numbers for TPC and mold counts are very low (TPC < 200; MC < 30) even after seven weeks of storage.

The low level of microbial growth during storage clearly indicated the long non-refrigerated shelf life. The sensory evaluation showed the potential marketing of this product. Also this product should be economical in cost since the raw materials were inexpensive, and lower-grade carcasses can be utilized; for example, even PSE meat or two-tone muscle is acceptable. It is expected that shredded pork can be a popular, long-term, commercial meat snack or food additive. In the past, most oriental products were handmade; therefore, they were always very time- and labor-consuming. With the designed modified process (modified autoclaving method) and the rapid advance of automation, meat floss can become a new product. The modified method saved approximately one-half of the original processing time (five hours or longer). In addition, the difference between other factors (raw material temperature and lard addition) didn't seem to have much influence on most sensory attributes; therefore, it suggests that the manufacture of meat floss is very flexible in relation to raw material and ingredients utilized. With today's advanced machinery techniques, it can be predicted that automated processing can solve the equipment problem and make the commercialized mass-production of the meat floss possible in the future.

References

Chang, S. F. and Huang, T. C. 1991. *Meat Sci.* 30:303–325.

Harris, D. H., Chaney, F. B. 1969. Human performance in industry. pp. 1–14. In: *Human Factors in Quality Assurance.* John Wiley & Sons, Inc., New York.

Lawrie, R. A. 1991. The storage and preservation of meat. pp. 152–172. In: *Meat Science.* Pergamon Press, New York.

Ockerman, H. W. 1985. *Quality Control of Post-Mortem Muscle Tissue.* Vol. 1. pp. 51.0–51.2 and 70.1–80.1. Department of Animal Sciences, The Ohio State University and the Ohio Agricultural Research and Development Center, Columbus and Wooster, Ohio, USA.

A Review of *Listeria monocytogenes* — A Pathogen That Likes Refrigerated Temperatures

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Abstract

The fact that *Listeria monocytogenes* can grow at refrigerated temperatures, which is often the technique that the meat industry uses to control pathogens, makes this microorganism a special problem for meat processors. Since this pathogen can be deadly and can cause abortions, it is receiving a lot of attention from a food-safety standpoint. Fortunately, non-pregnant healthy adults are fairly resistant. Also, pasteurization and chemical additives are fairly effective at protection, but a rapid analytical technique for identification would be extremely helpful in combating this troublemaker for meat products.

Review

There are several types of *Listeria* spp., but the most pathogenic one is *Listeria monocytogenes*. Others include *Listeria innocua, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii, Listeria grayi,* and *Listeria murrayi. Listeria monocytogenes* is responsible for 98% of the human listerosis cases identified. This organism was first described in 1923, and the first human listeriosis was reported in 1929. This pathogen has been shown to be a problem in more than 50 mammal types, and the overall mortality rate can be from 10 to 70%. *Listeria* may be found in decaying vegetation, soil, animal feces, sewage, silage, water, and contaminated food, including meat. It is obvious that this contamination can occur in most any food item and is particularly a problem with perishable items. *Listeria* spp. is fairly common in the household and can be found in 47% of household supplies. Of this 47%, *Listeria monocytogenes* makes up about 41% of this population. The highest percentage is found on dishcloths and wash-up brushes, and so these areas need special attention in the cleanup operation. *Listeria* is also moderately common in meat products, with about 0.5% beef jerky containing this microfile, about 1.3% large-diameter cooked sausage may contain Listeria, 2.3% of cooked poultry, 2.8% of corned beef and cooked roast beef, 4.2% of small-diameter cooked sausage, and 6% of ham slices and luncheon meat. It is pertinent that we be aware that meat products can be a problem and that special precautions should be taken.

Listeria monocytogenes is especially harmful to people with AIDS, alcoholism, diabetes, cardiovascular disease, renal transplants, and corticosteroid therapy. A major area of concern is abortion as fetuses are highly susceptible. Fortunately, nonpregnant healthy adults are highly resistant. This translates into a variable dose rate to cause infection. Epidemiology data indicates that consumption of fewer than a thousand total organisms in milk by *susceptible* people may cause the disease. However, a study with *healthy* primates indicates that consumption of as high as 100 million cells was required to cause the disease. USDA product recalls due to *Listeria* were one in 1997, five in 1998,

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and nine in 1999, suggesting that this problem is not going away and, in fact, may be increasing. The Center For Disease Control has suggested that in 1996 for every 100,000 individuals, *Listeria* was responsible for 0.4 cases; in 1997, 0.5 cases; and in 1998, 0.5 cases. In 1996–97 this resulted in 77 cases in the United States, with 15 deaths. Therefore, the problem can be serious, and special precautions should be taken.

Listeria monocytogenes likes warm temperatures of about 50°F but can also survive and grow at refrigerated temperatures, which makes it a special problem. For example, at 40°F, the germination period (time required to double in number) is approximately three days. At 32°F, the germination time would be 62 to 131 hours, and at 40°F, it has been estimated at 18 to 30 hours, all of which is a problem for products that are protected by refrigeration.

Government tolerance levels permitted in food are as follows: For the United States, it is zero cells for sampling unit, and for Canada, the European community, and Australia, it is less than 100 units per gram. So in all cases, the permissible legal level is very, very low. How can the product be protected — by paying attention to raw materials, educating employees, cleaning floors and equipment properly, and handling cleanup equipment and material properly.

Cooking is always a critical control point in the production of this type of product and particularly where *Listeria* is involved, but time and temperature will be different for each individual product. Smokehouse and cook chambers should also receive special attention to make sure they are not a contaminating source. Cooking temperature to kill *Listeria* is 158°F for two minutes, and this will destroy this pathogen. Frankfurters should be cooked to 160°F. This will result in a three-log reduction. Initial contamination as well as cooking temperature is important.

Continuous training and monitoring is essential for all microbial problems. This should be concentrated particularly in the area of hygiene and product-handling procedures. Areas that need special attention are personnel entrances, lift-truck entrances, and work areas around equipment. Equipment should also be designed so it is easy to maintain, clean, and sanitize and even non-food contact surfaces should receive special attention particularly if they are in the proximity of food contact surfaces which, are of course, absolutely critical from a sanitation perspective. Following appropriate pasteurization techniques along with using chemical additives are needed to help the food processor fight against *Listeria monocytogenes*. A more closed-system environment, with more automation and improved sanitation techniques, is very critical as is a rapid analytical tool for detecting this microorganism in a short period of time. The meat industry has recognized the problem and is taking steps to make sure your meat supply is safe. Handling after it leaves the meat plant, however, is still an area that needs added emphasis.

Some Physicochemical Changes in Catfish Muscle as Influenced by Egg White and Tumbling

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Abstract

Egg white (1%) and intermittent tumbling (20 minutes work and 40 minutes rest) (12 hours at 6°C) positively influenced the physiochemical properties of fish muscle tissue when compared with non-treated tissue. Salt-soluble protein extraction allowed the production of a fish log out of fish pieces. With these treatments, little proteolysis and a satisfactory flavor were noted and maintained during extended refrigerated storage.

Introduction

There have been a number of research projects in the fishery area investigating the effects of protein hydrolysis on the functionality of muscle foods, i.e., emulsion, gelling, and binding properties. For example, the quality of a cooked meat gel (kamaboko) is highly related to its elasticity, and if a poor elastic gel is produced, it often loses its commercial value due to the proteolysis taking place during cooking (Makinodan *et al.*, 1985; Boye and Lanier, 1988). Unlike terrestrial animal tissue, fish muscle has remarkably high indigenous proteases which immediately start to break down the proteins after the fish are harvested and during processing or improper handling, storage, and cooking (Aksnes, 1989; Morrissey et al., 1993). These proteases are active either at low or high temperatures and are responsible for flakiness, non-adhesive attributes, and weak gel-forming ability of fish muscle. Non-fish proteins or binders were incorporated to produce surimi-type fish products to improve gelling and binding properties. But little is known about the relationships between the physicochemical properties of non-fish protein and the textural properties of restructured fish products (Aksnes, 1989; Chung and Lee, 1990; and Yu, 1992). However, the literature on surimi and protein functionality contains many reports (Babbitt and Reppond, 1988; Hastings *et al.*, 1990; and Chan *et al.*, 1992). The limitations for the use of various fish species for surimi production are usually due to higher proteolytic activity and the degradation of the proteins, resulting in a poor product. This problem has been illustrated for many fish species (1.2). It is reported that the incorporation of protease inhibitors such as egg white, wheat or potato starch, plasma hydrolysate, whey proteins, and soy protein isolate would be necessary to prevent proteolysis and maintain gel-forming ability of surimi products. (Aksnes, 1989; Lee, 1986).

The tumbling technique, which has been widely used in the red-meat industry, is a physical operation in which meat pieces are subjected to physical forces to improve quality characteristics by accelerating the curing process. It relies on gravitational impact and abrasion against other meat pieces to disrupt muscle fibers and extract the myofibrilar proteins that are necessary for binding meat sections together. Subsequently, molding or placing the meat fragments into a casing and cooking will produce the final tumbled and re-

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formed meat product (Yetim, 1993; Yetim and Ockerman, 1995). To satisfy the growing demand for processed seafood products (Chung and Lee, 1990), new types of restructured fish products should be considered since there is currently very limited choice of these types of products. There is also minimal information on the indigenous proteases and construction elements of restructured fish products and their cooking responses, and there has been no study regarding tumbling of fish muscle for reforming purposes.

The objectives of this research were to evaluate physicochemical changes of fish muscle during processing, to monitor the activity of indigenous proteases (*in situ*), to observe the effects of a non-fish protein incorporation and a tumbling technique on the water-holding properties and texture of fish muscle.

Materials and Methods

Fresh channel catfish fillets were first diced (approximately 3 x 3 x 2 cm), and subjected to the following: 1) nontumbled, no egg white added (control); 2) tumbled, no egg white added; 3) nontumbled, egg white added; and 4) tumbled, egg white added. The ingredients (2% salt, 1% egg white powder [in egg-white batches], 1% sucrose, 0.5% white pepper, 0.1% garlic, 0.25% sodium tripolyphosphate, 0.01% nitrite, and 0.01% natural lemon flavor) were added into the product on a fish weight basis, and the intermittent tumbling (20 minutes work, 40 minutes rest) was done for 12 hours at 6°C on the tumbling batches while storing the nontumble batches in the same place for the same time.

Degree of Proteolysis

Degree of proteolysis was determined as described by Aksnes (1988) with a slight modification. The trichloroacetic acid (TCA) soluble nitrogen concentration was expressed as a percentage of the original nitrogen in the sample and was considered as degree of proteolysis.

Effective Protein Hydrophobicity (EPH)

The EPH values of the protein samples from the myofibrilar fraction of the fish muscle were measured by a heptane binding method based on the reports by Mohammadzadeh-k *et al.* (1969) and Mangino *et al.* (1985) using a Hewlet-Packard 5890A (Starrod Avendale, Pa.) gas chromatograph (**GC**) equipped with a hydrogen-flame ionization detector. A standard curve was prepared using an undecane, n-heptane, and nonane solutions to compute the effective protein hydrophobicity values which were fully described by Yetim (1993).

Expressible Moisture

Expressible moisture was determined by quantifying the amount of water expressed on three layers of filter paper (Whatman #1) upon compression by the Instron testing machine (Model 1000, Instron Co., Canton, Maine) as described by Lee and Chung (Lee and Chung, 1989). It was reported in percentage of total moisture. The total gel moisture content of the sample was determined by the oven method (Ockerman, 1985).

Total N and pH

Total nitrogen was determined by kjeldahl procedure, and a pH meter (Radiometer, PHM 22 Copenhagen, Denmark) was used for pH measurements as described by Ockerman (1985).

Experimental Procedure and Statistical Analysis

Fresh (zero time) control and the four treatment groups were sampled after the tumbling process. The experiment consisted of a 1 + (2 x 2) factorial block design (fresh, tumbling, and egg white) with five replications. Differences and comparisons of treatment means and correlation were determined using the SAS program (SAS, 1985).

Results and Discussion

The changes in proteolysis, effective protein hydrophobicity (EPH), and expressible moisture (EM) values due to tumbling and egg-white incorporation are shown in Figures 1, 2, and 3. There were significant ($P < 0.05$) differences between fresh and processed samples in terms of proteolysis, EPH, and EM. The tumbling did not make a significant difference; another statistical analysis was conducted by combining the related observations, where appropriate. The effects of either tumbling or egg-white addition on raw fish were ob-

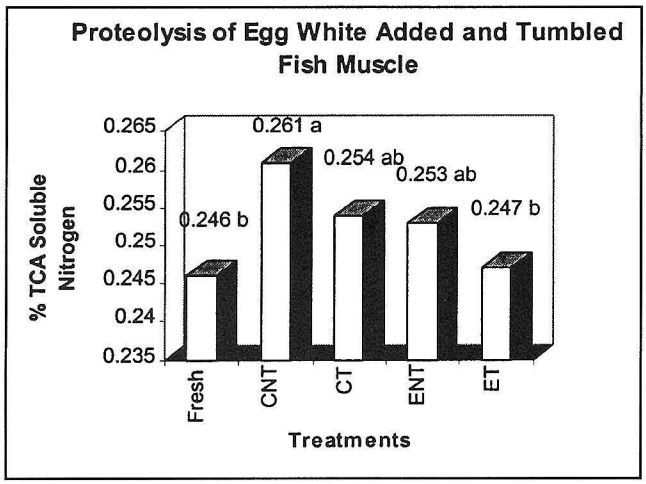

Figure 1. Changes in the proteolysis (% Trichloroacetic acid-soluble nitrogen) with tumbling and egg white in fresh or processed fish muscle. Fresh: Zero Time Condition, CNT: Control-NonTumble, CT: Control-Tumble, ENT: Egg White-NonTumble, ET: Egg White-Tumble. [a,b] Means with the same letters are not significantly different (P > 0.05).

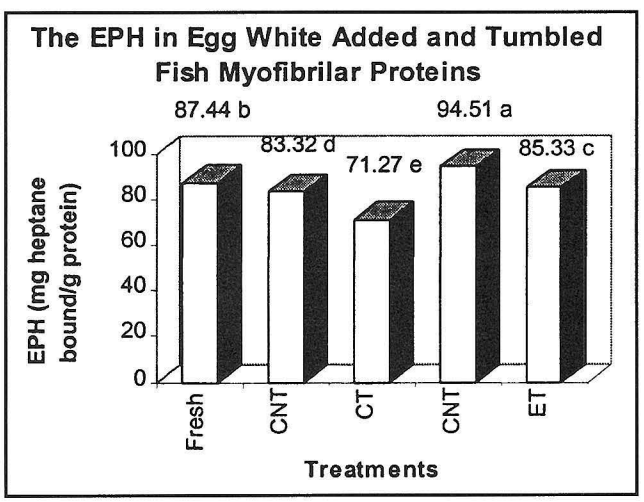

Figure 2. Changes in degree of (EPH) effective protein hydrophobicity (mg heptane/g protein) of proteins with tumbling and egg white in fresh, or processed fish muscle. Fresh: Zero Time Condition, CNT: Control-NonTumble, CT: Control-Tumble, ENT: Egg White-NonTumble, ET: Egg White-Tumble. [a,b,c,d,e] Means with the same letters are not significantly different (P > 0.05).

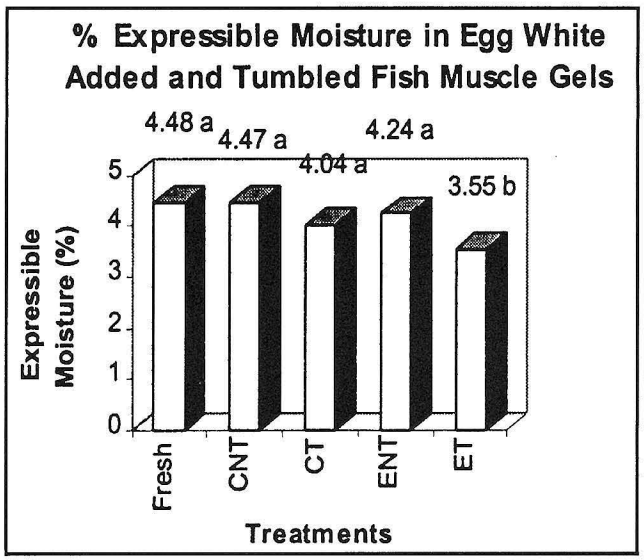

Figure 3. Changes in the amount (%) of expressible moisture with tumbling and egg white in processed fish muscle gels. Fresh: Zero Time Condition, CNT: Control-NonTumble, CT: Control-Tumble, ENT: Egg White-NonTumble, ET: Egg White-Tumble. [a,b] Means with the same letters are not significantly different (P > 0.05).

served. The results can be seen in detail in Table 1. The data presented in this table are the mean values of the selected parameters from pooled data of each treatment in which fresh (zero-time) observations are not included. Egg white had a significantly lowered proteolysis and EM while yielding higher EPH as compared to no-egg white added control groups. On the other hand, tumbling did not affect the degree of proteolysis, but it significantly decreased EM and EPH values of the fish proteins.

Proteolysis

A slight but significant proteolysis took place in the control group during processing compared to the zero-time (fresh) analysis. Egg-white-added-and-tumbled products had slightly lower proteolysis value, but it was not significant. However, it can be stated that egg white plus tumbling action may have prevented further proteolysis in the fish product (Figure 1). Additionally, as can be seen in Table 1, egg-white-added samples had lower proteolysis compared to the no-egg-white-containing samples. However, tumbling did not significantly

affect the rate of proteolysis though it showed a lower proteolysis value (Table 1). There are many reports that support this finding that egg white either prevents or reduces the proteolysis in fish muscle although no study was found with tumbling (Chung and Lee, 1990; Haga *et al.*, 1980; Hamann *et al.*, 1990). In general, all the treatments showed a fairly low proteolysis, which may have been affected by the salt content used in this experiment.

Inhibition of the proteolytic activity with high percentage of NaCl in fish muscle was reported by Noda *et al.* (1982). Also, it was determined that endogenous proteolytic enzymes were susceptible to NaCl (Fik *et al.*, 1985). They reported that the inhibitory effect of NaCl on fish enzymes increased with increasing NaCl concentration, but this effect decreased with increasing hydrolysis time.

Table 1. The Influence of Egg White (EW) on Proteolysis, Effective Protein Hydrophobicity (EPH), and Expressible Moisture (EM) of Processed Fish Muscle.

Parameter[1]	No-EW Added	± SE[6]	EW Added	±SE
Proteolysis[3]	0.258[a]	0.004	0.250[b]	0.003
EPH[4]	77.295[b]	2.277	89.920[a]	1.734
EM[5]	4.260[a]	0.219	3.896[b]	0.207
Parameter[2]	**Non-Tumble**	**± SE**	**Tumble**	**± SE**
Proteolysis[3]	0.256	0.003	0.251	0.004
EPH[4]	88.915[a]	2.114	78.300[b]	2.657
EM[5]	4.332[a]	0.208	3.733[b]	0.209

1 Tumbling data were combined.
2 Egg-white data were combined.
3 % Trichloroacetic acid soluble nitrogen.
4 Effective protein hydrophobicity by heptane binding (mg heptane/g protein).
5 % Expressible moisture in muscle gel.
6 SE = Standard Error.
a,b Means with the same superscript letters in a row are not signicantly different (P > 0.05).

In this experiment, 1.0% egg white reduced the proteolytic activity during processing (Table 1 and Figure 1), although there was a relatively small amount of proteolysis. However, Haga *et al.* (1980) reported that the use of 3.0% dried egg white reduced the proteolysis and would improve the gel strength of surimi made from Pacific whiting. Likewise, Chung and Lee (1990) observed the superiority of 3.0% egg white to improve the textural and functional properties of whiting surimi over equal levels of some other protein concentrates. At 2.0% addition of egg-white proteins, gel strength of the fish muscle was significantly increased, and it was also postulated that using a protease inhibitor would be necessary to improve textural quality of some fish products (24).

Effective Protein Hydrophobicity (EPH)

There were very significant alterations in the EPH caused by the treatments compared to fresh fish. The EPH was significantly increased with egg white compared to fresh or the other control group. However, EPH was significantly reduced by the tumbling process, which included either tumbling alone or tumbling plus egg-white treatment (Table 1 and Figure 2). This result was interesting because the influence of tumbling on EPH was in the negative direction compared to the egg-white treatment, which was a positive influence. Li-Chan *et al.* (1987) reported that unheated cod-fish muscle samples had the highest protein hydrophobicity, but showed relatively low gel strength compared to other species. It has been postulated that increased hydrophobicity is generally related to the amount of exposed hydrophobic groups of the proteins in the food systems (LeBlane and LeBlane, 1992). Also, there was a significant positive correlation between pH and EPH. Increasing the pH of the product yielded more protein hydrophobicity, which is thought to be related to a number of other functional properties of food proteins (LeBlane and LeBlane, 1992; Kato and Hakai, 1980).

Expressible Moisture (EM)

As shown in Figure 3, a significantly low EM was observed in the egg-white-added-plus-tumbled treatment when compared to fresh and control groups, but there were no significant differences between the control and other treatments for EM. That is, egg white did not significantly reduce expressible moisture although the percent value was lower. However, pooled data in Table 1

showed that EM was lowered with either egg white or tumbling, although the only significance was found with tumbling plus egg white with unpooled data (Figure 3). Similar results for EM were also reported by Chung and Lee (1990); however, the compressive force of the surimi gel samples was significantly increased with egg-white addition. A negative significant correlation was noted between pH and EM; this result would be expected because of the relation between the isoelectric pH of muscle proteins and water-holding capacity (Ockerman, 1983).

Conclusions

Results indicate that either the separate or the combination of egg-white addition and tumbling significantly altered physicochemical properties of fish-muscle tissue studied in this experiment. Egg-white incorporation increased effective protein hydrophobicity and decreased proteolysis and expressible moisture. Tumbling, on the other hand, decreased proteolysis, EM, and EPH as compared to the nontumbled control groups. Egg white plus tumbling had also a reducing effect on proteolysis and EM in the processed fish muscle. In general, all treatments resulted in a fairly low proteolysis which may be due to the salt content which has reportedly caused a reducing effect on proteolytic activity in fish muscle. The tumbling action and egg-white addition significantly influenced effective hydrophobicity. It is interesting that the influence of tumbling on EPH was in the negative direction, while egg white showed the reverse outcome. Also, increasing the pH of the product increased protein hydrophobicity, which will affect the protein functionality. A significant positive correlation (0.79) occurred between pH and EPH while a significant negative correlation (-0.59) was noted between pH and EM. Therefore, it might be suggested that tumbling and egg-white addition may be utilized to increase the functional properties of fish proteins during processing.

References

Abide, G. P., Hearnsberger, J. O. and Silva, J. L. 1990. *J. Food Sci.* 55:1747–1748.

Aksnes, A. 1988. *J. Sci. Food Agric.* 44:263–271.

Aksnes, A. 1989. *J. Sci. Food Agric.* 49:225–234.

Babbitt, J. K. and Reppond, K. D. 1988. *J. Food Sci.* 53:965–966.

Boye, S. W. and Lanier, T. C. 1988. *J. Food Sci.* 53:1340–1342, 1398.

Chan, J. K., Gill, T. A. and Paulson, A. T. 1992. *J. Food Sci.* 57:906–912.

Chung, K. H. and Lee, C. M. 1990. *J. Food Sci.* 55:972–975, 988.

Fik, M. Sypniewska, L., and Pham, T. N. 1985. *Acta Alimentaria Polonica.* 11:305–312.

Haga, H., Shigeoka, R. and Yamauchi, T. 1980. U.S. Patent 4,207,354, Jun. 10.

Hamann, D. D., Amato, P. M., Wu, M. C. and Foegeding, E. A. 1990. *J. Food Sci.* 55:665–669.

Hastings, R. J., Keay, J. N. and Young, K. W. 1990. *J. Food Sci. & Technol.* 25: 281–294.

Kato, A. and Nakai, S. 1980. *Biochim. Bioph. Acta.* 624:13–20.

LeBlane, E. L. and LeBlane, R. J. 1992. *Food Chem.* 43:3–11.

Lee, C. M. 1986. *Food Technol.* 40 (3):115–124.

Lee, C. M. and Chung, K. H. 1989. *J. Texture Studies.* 20:363–377.

Li-Chan, E., Nakai, S. and Wood, D. F. 1987. *J. Food Sci.* 52:31–41.

Makinodan, Y., Toyohara, H. and Niwa, E. 1985. *J. Food Sci.* 50:1351–1355.

Mangino, M. E., Fritsch, D. A., Liao. S. Y., Fayerman, A. M. and Harper, W. J. 1985. *NZ J. of Dairy Sci. and Technol.* 20: 103–107.

Mohammadzadeh-k, A., Feeney, R. E. and Smith, L. M. 1969. *Biochim. Biophys. Acta.* 194:246–255.

Morrissey, M. T., Wu, J. W., Lin, D. and An, H. 1993. Effect of food grade protease inhibitors on autolysis and gel strength of surimi. 1993-IFT Ann. Mt. Tech. Prog. Abst. 346, pp 89, July 10–14, Chicago, IL.

Noda, M., Van, T. V., Kusakabe, I. and Murakami, K. 1982. *Agric. and Biol. Chem.* 46:1565–1569.

Ockerman, H. W. 1983. *Chemistry of Meat Tissue.* Department of Animal Sciences, The Ohio State University, Columbus, Ohio.

Ockerman, H. W. 1985. *Quality Control of Muscle Tissue.* Department of Animal Sciences, The Ohio State University, Columbus, Ohio.

Reppond, K. D. and Babbitt, J. K. 1993. *J. Food Sci.* 58:96–98.

SAS. 1985. *SAS User's Guide: Statistics.* 5th Ed. SAS Institute, Inc., Raleigh, N.C.

Yetim, H. 1993. *Biochemical and Structural Alterations of Restructured Fish Muscle as Influenced by Egg White, Tumbling, and Storage Time.* Dissertation. Department of Animal Sciences, The Ohio State University, Columbus, Ohio.

Yetim, H. and Ockerman, H. W. 1995. *J. Aqua. Food Prod. Technol.* 4:65–78.

Yu, S. Y. 1992. *ASEAN Food J.* 7 (1):51–52.

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